Supplementary figure 1

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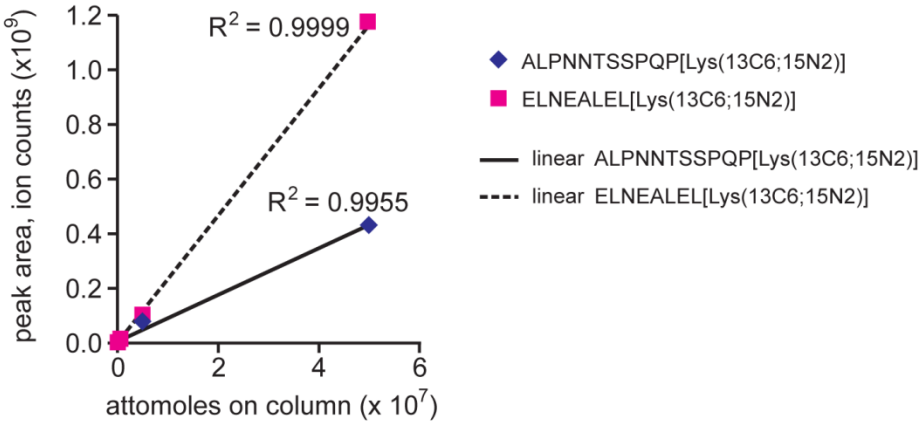

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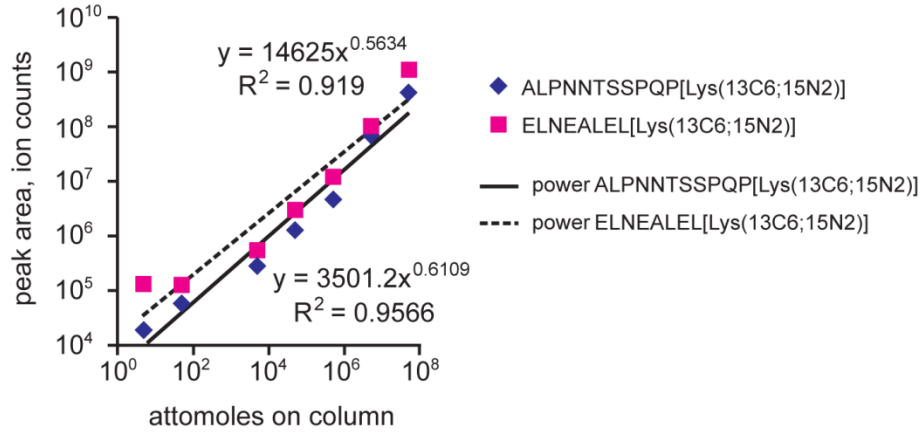
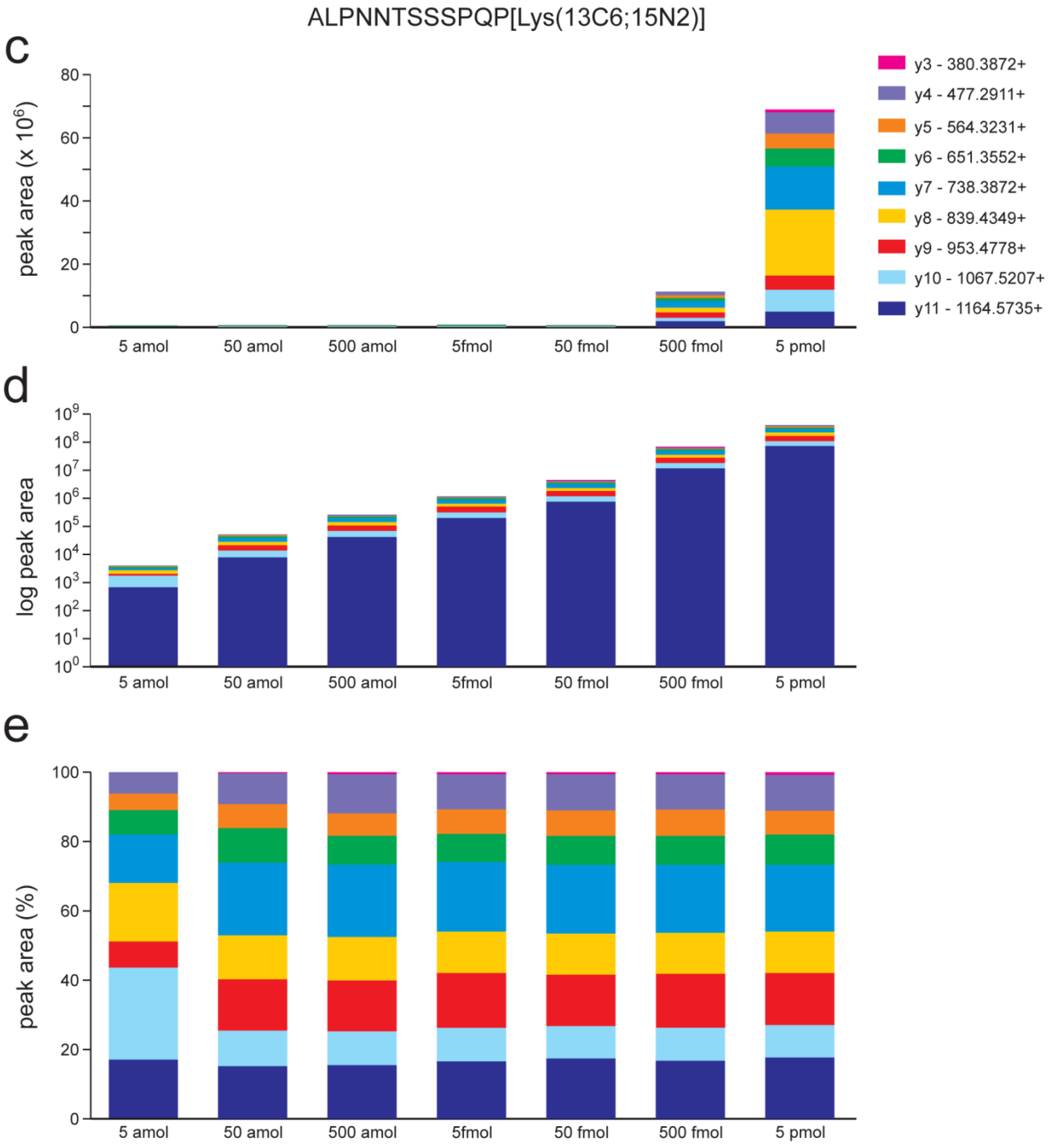
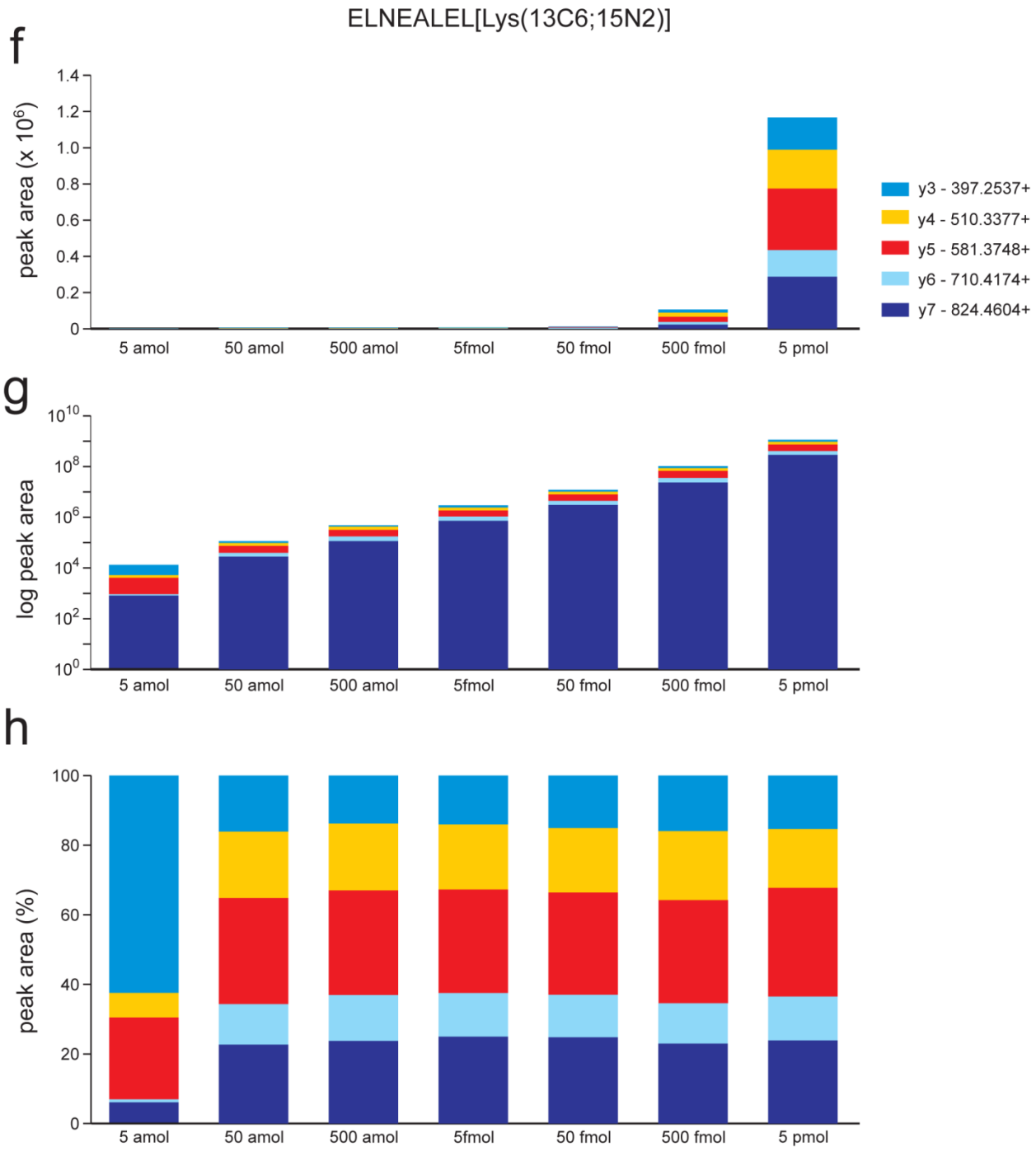


Supplementary figure 1



Supplementary figure 1

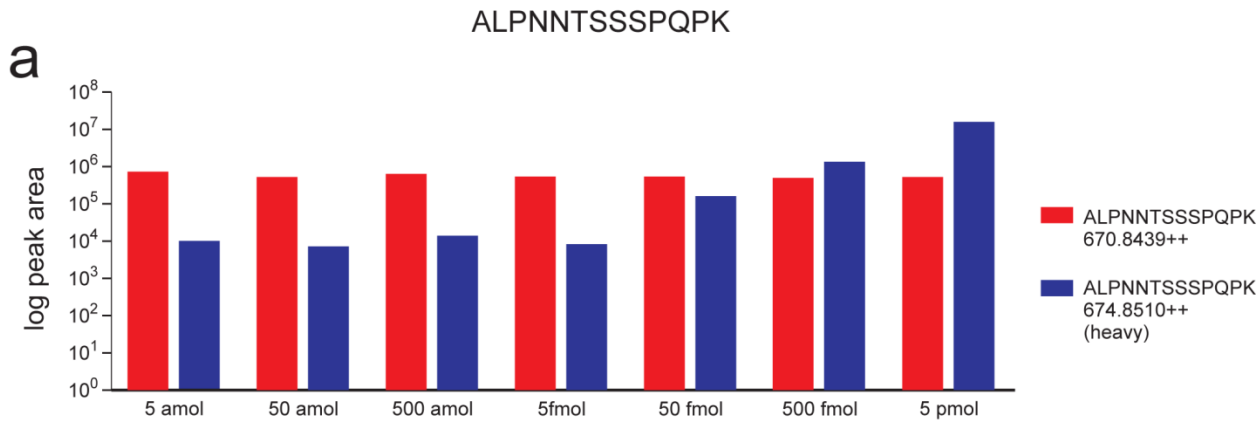


Supplementary Figure 1. Limit of quantitation (LOQ) and limit of detection (LOD) were determined for SIL peptides in buffer solution. Presented in this figure are the regression analysis to demonstrate linearity of peak areas in response to increasing amount of spiked-in protein, and peak areas as extracted by Skyline software, mapped in absolute amount, on logarithmic scale, and normalized to total intensity. The normalized intensities most accurately reflect relative ion fragment ratios regardless of absolute amount.

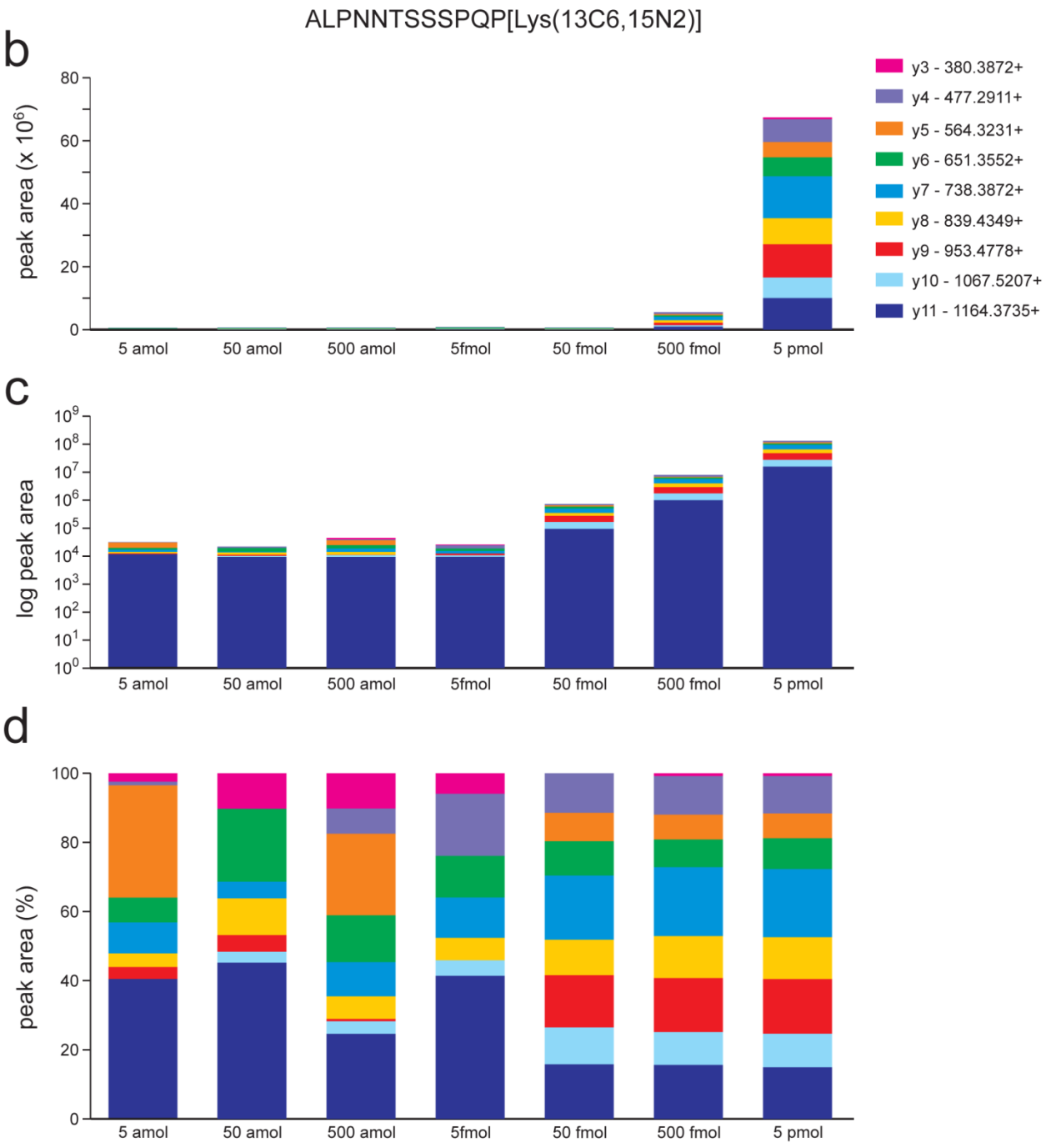
Linear regression analysis of both SIL peptides shows a linear response over 6 orders of magnitude (**a**), while power regression of log-transformed data shows linear response over 4 orders of magnitude (**b**).

Absolute (**c**), log-transformed (**d**), and normalized (**e**) peak areas and contributing ion ratios from TP53 peptide ALPNNSSSPQPK are graphed from 5 amol to 5 pmol amounts spiked into ammonium bicarbonate buffer. Same peak area measurements are demonstrated for TP53 peptide ELNEALELK respectively in panels (**f**), (**g**), and (**h**).

Supplementary figure 2



Supplementary figure 2



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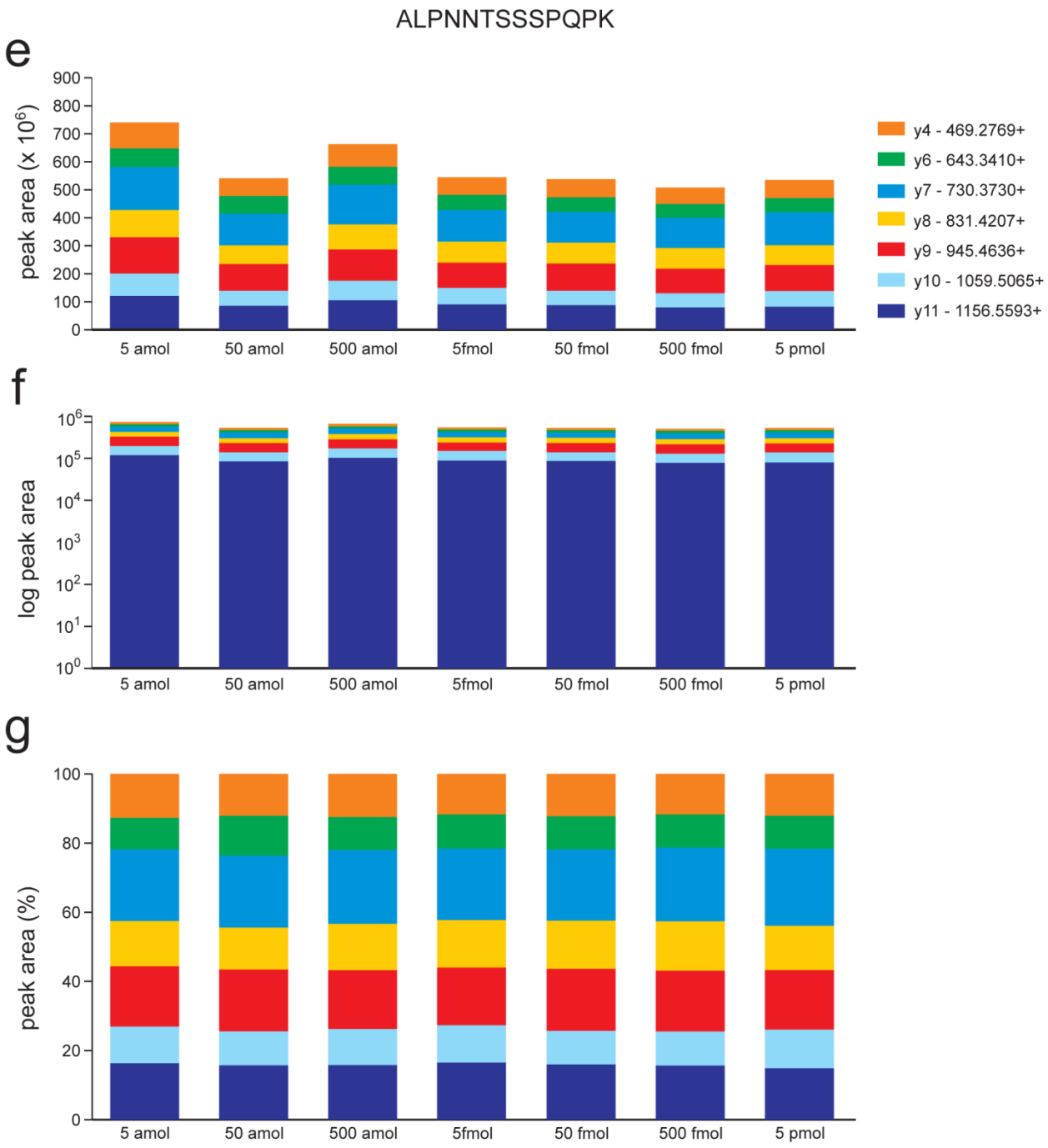

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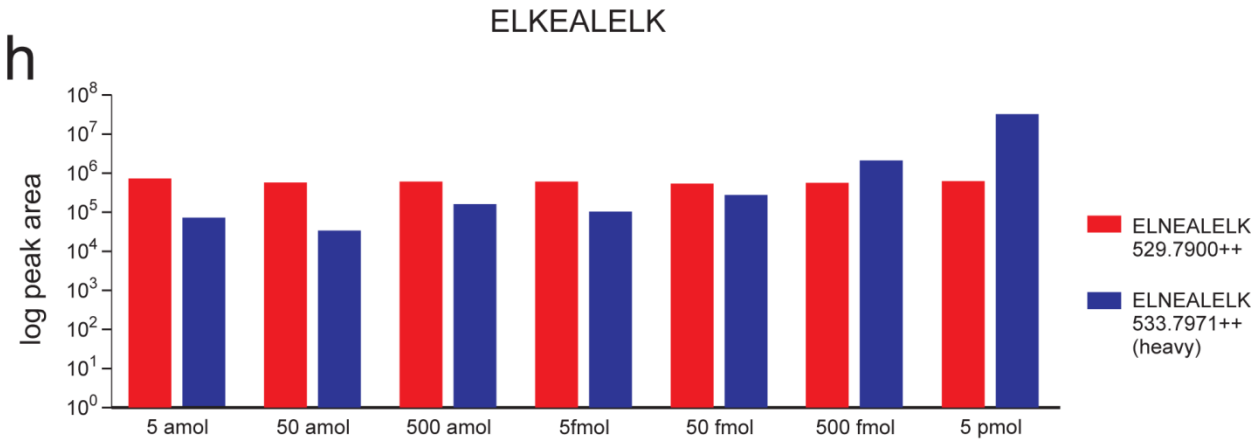

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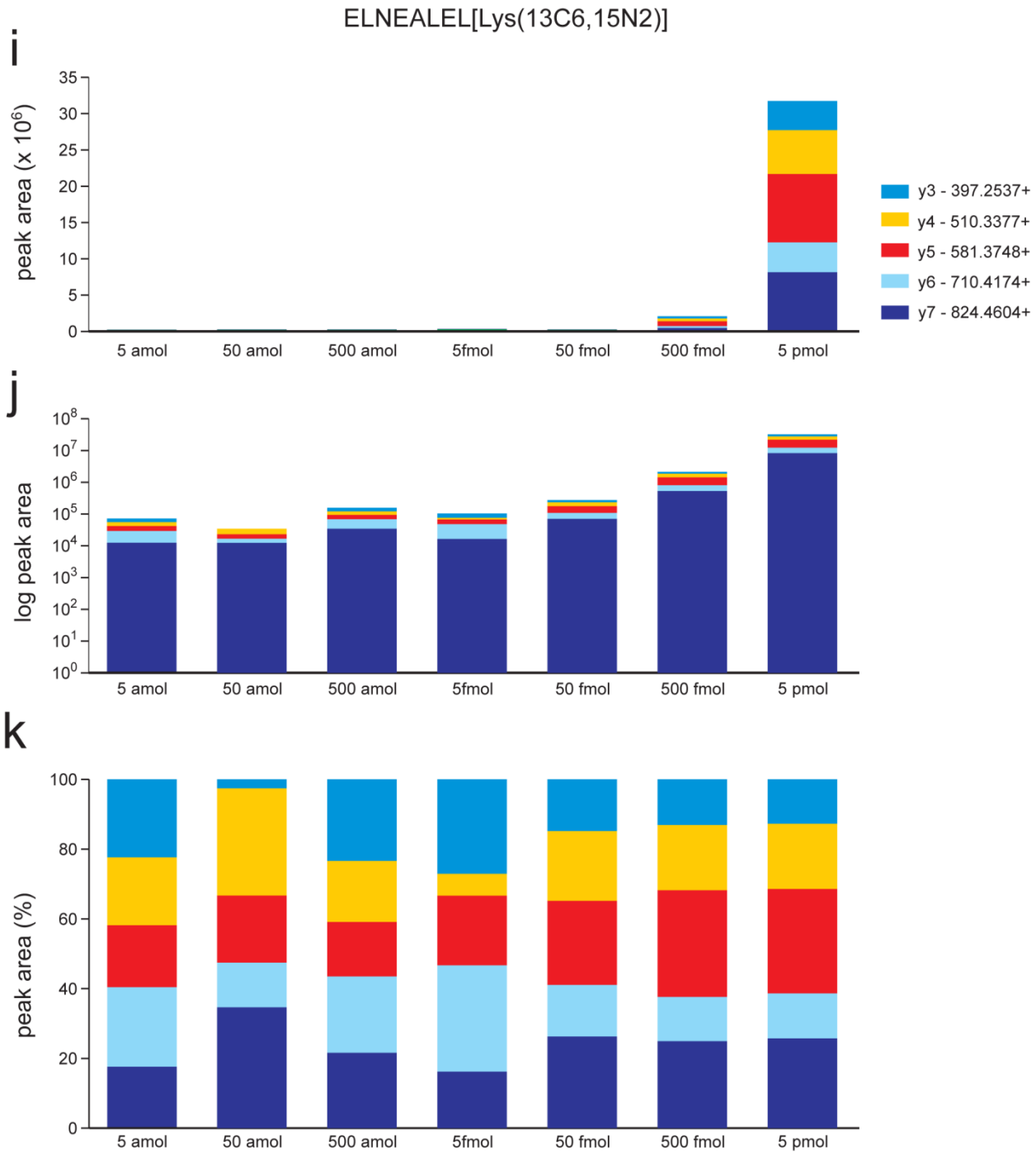

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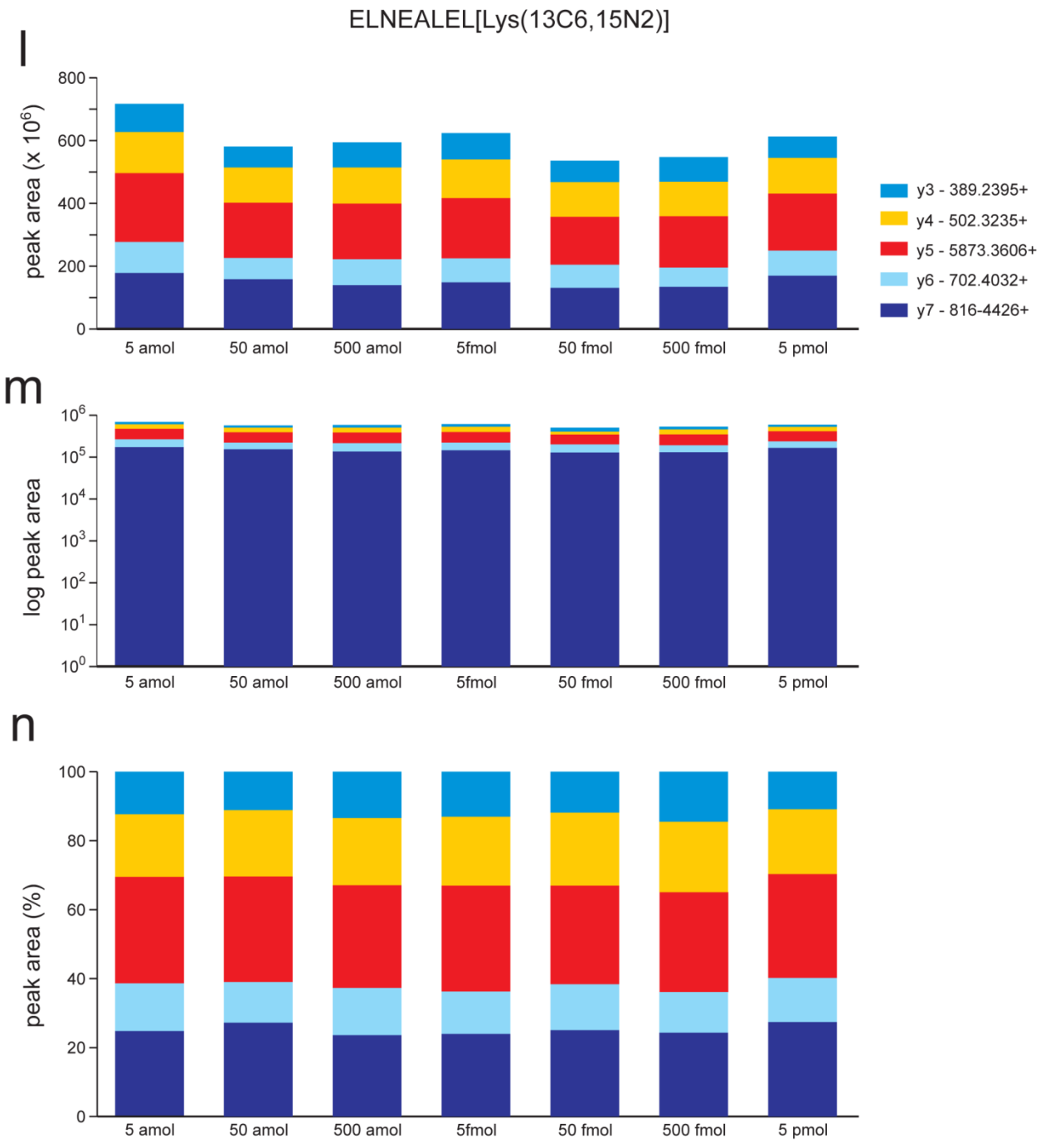
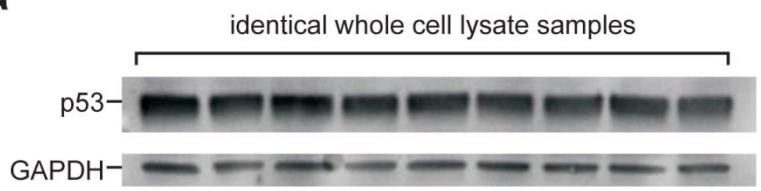


Supplementary Figure 2. Limit of quantitation (LOQ) and limit of detection (LOD) were determined for SIL peptides within a complex background of whole cell extract (WCE) using TP53 peptides ALPNNSSSPQPK and ELNEALELK. Panel (**a**) shows relative peak area ratios of an endogenous TP53 peptide ALPNNSSSPQPK extracted from WCE and peak areas for its heavy cognate ALPNNTSSSPQP[Lys(13C6; 15N2)], which was added in indicated amounts. Panels (**b**), (**c**), and (**d**) show absolute, log-transformed, and normalized peak area intensities and ion fragment ratios for the heavy peptide. Panels (**e**), (**f**), and (**g**) show absolute, log-transformed, and normalized peak area intensities and ion fragment ratios for the endogenous peptide.

Panel (**h**) shows relative peak area ratios of the endogenous peptide ELNEALELK found in WCE and peak areas for its heavy cognate ELNEALEL[Lys(13C6; 15N2)], which was added in indicated amounts. Panels (**i**), (**j**), and (**k**) show absolute, log-transformed, and normalized peak area intensities and ion fragment ratios for the heavy peptide. Panels (**l**), (**m**), and (**n**) show absolute, log-transformed, and normalized peak area intensities and ion fragment ratios for the endogenous peptide.

Supplementary figure 3

a

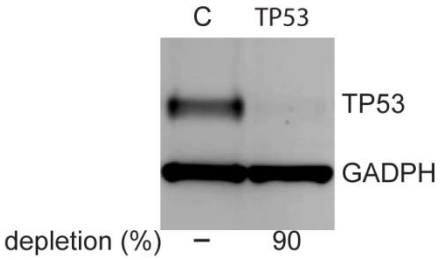


b

Western blot	CV (%)	SRM peptide	CV (%)
TP53	16.9	ALPNNTSSSPQPK	16.8
		ELNEALELK	12.3
GAPDH	38.2	IISNASCTTNCLAPLAK	8.7
		VPTANVSVVDLTCR	5.4

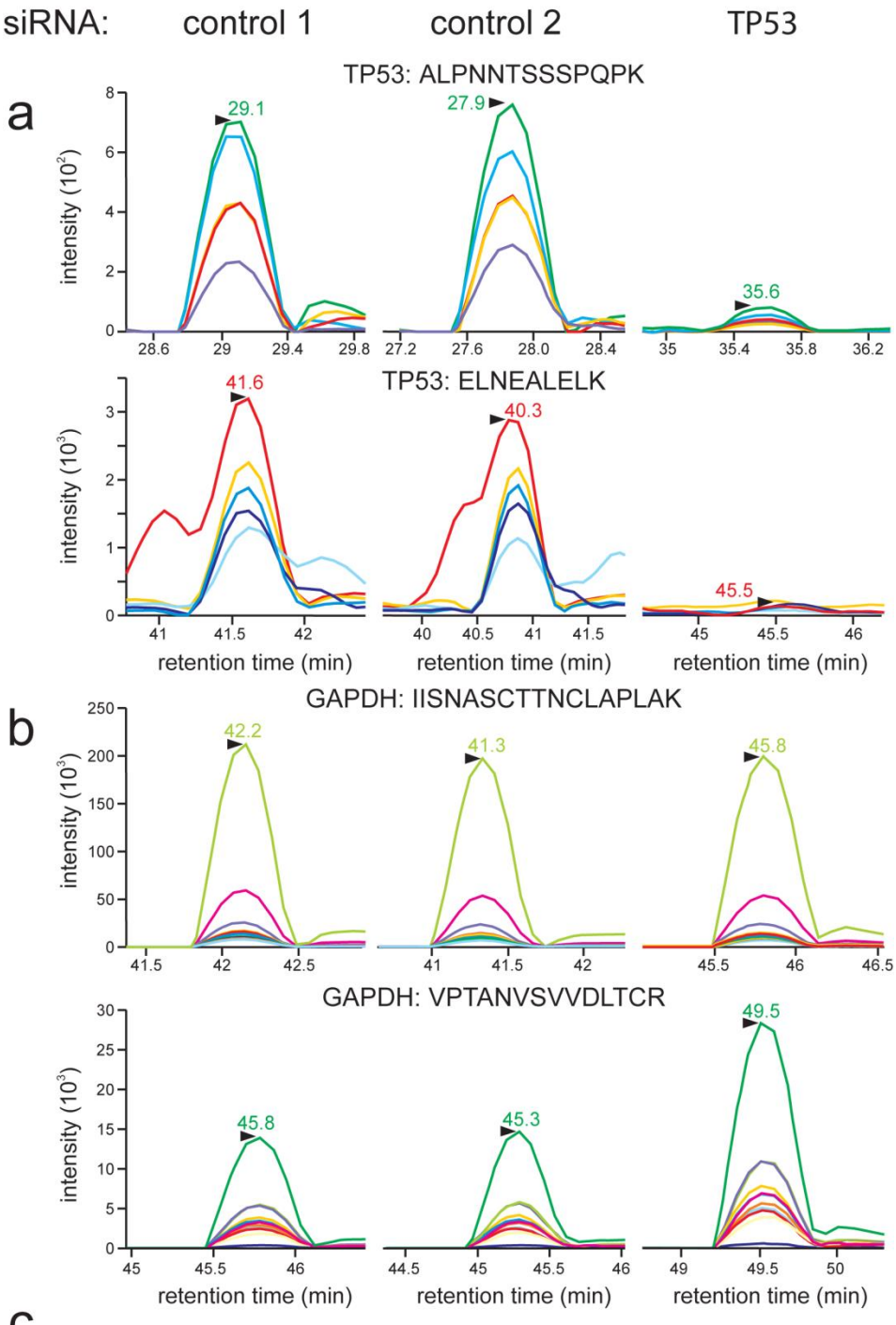
Supplementary Figure 3. Replicate analysis to determine the coefficient of variation (CV) for protein detection by Western blot or SRM. (**a**) Aliquots of 50 μg of total protein from human embryonic kidney (HEK-293T) cells were loaded in replicate lanes prior to electrophoresis and Western blot detection with anti-TP53 and anti-GAPDH antibodies (see Methods). In parallel, 6 technical replicates of trypsin-digested whole cell lysate from HEK-293T cells were analyzed by SRM as illustrated in **Figure 2** to detect and quantify TP53 and GAPDH. (**b**) CV values were determined for Western blot analyses by quantifying band intensities using Image Quant software, and by peak areas as determined by Skyline for SRM analyses.

Supplementary figure 4



Supplementary Figure 4. Western blot verification of TP53 depletion from human cells, prior to adding SIL peptides to samples. Cells were transfected with *TP53*-specific or control (C) siRNAs prior to preparing whole cell extracts. TP53 and GAPDH were detected by Western blot analysis. Band intensities for TP53 versus GAPDH were normalized against the blot background, then used to estimate percent TP53 depletion as a function of siRNA dose (**a**).

Supplementary figure 5

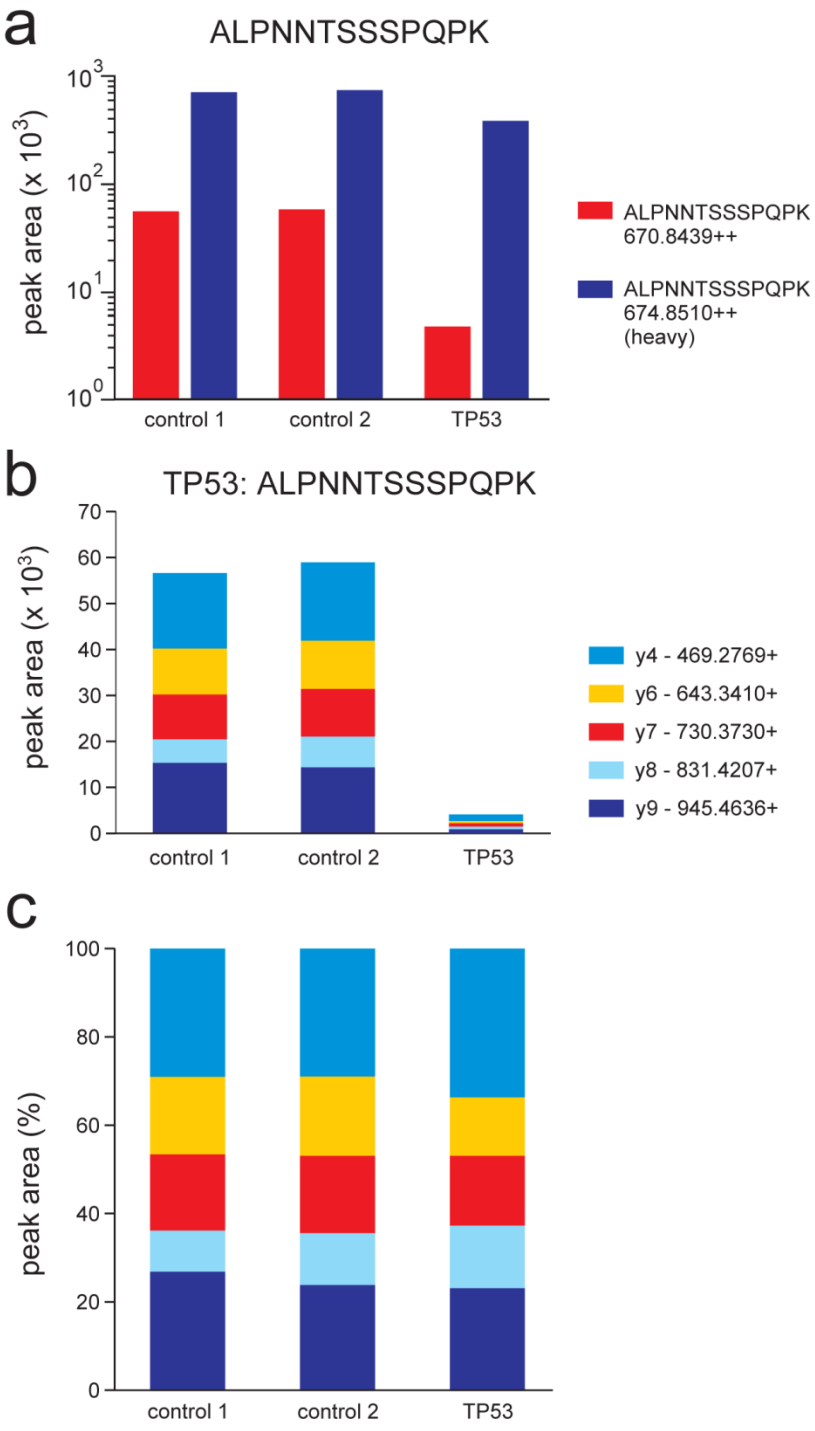


c

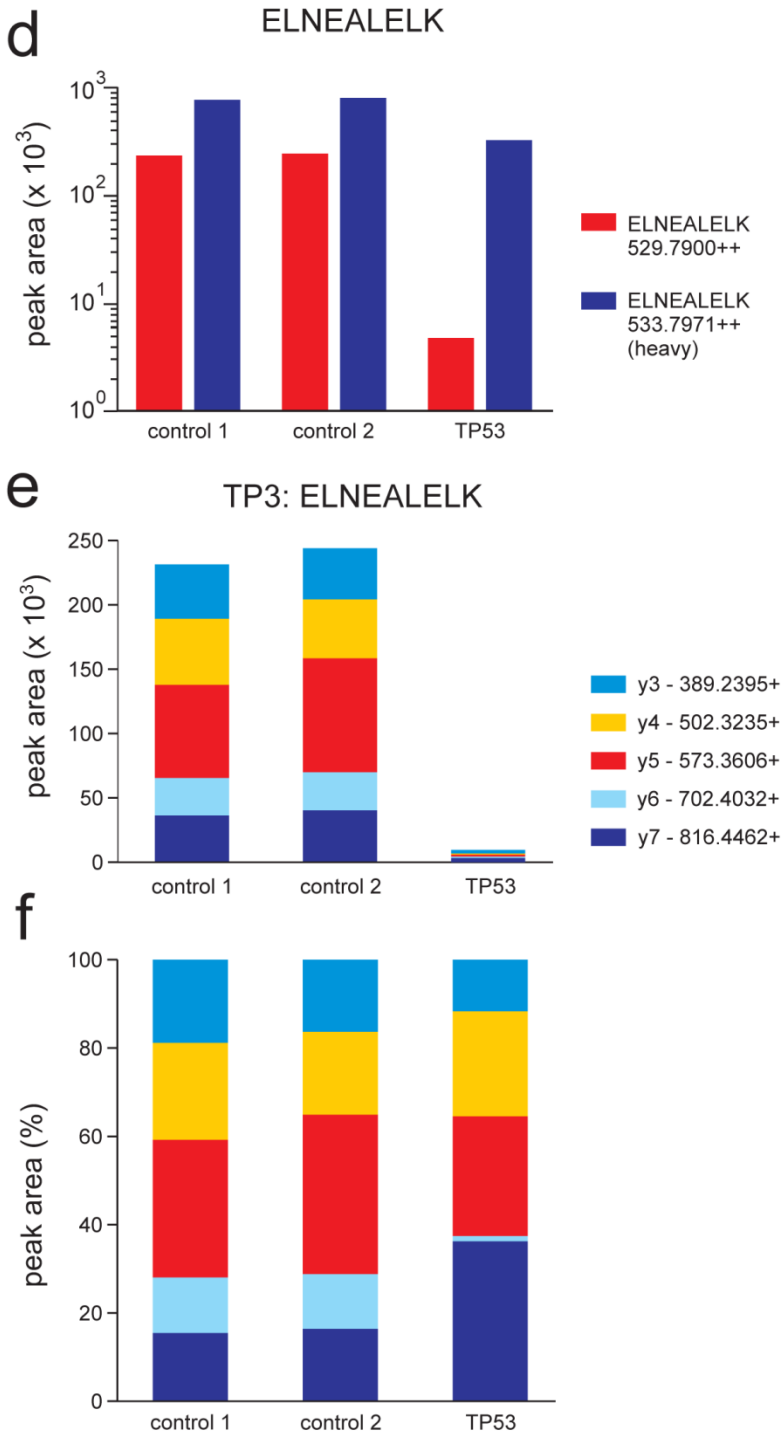
TP53 peptide	/	control peptide	TP53 depletion (%) control 1	control 2
ALPNNTSSSPQPK	/	ALPNNTSSSPQPK	86	89
ALPNNTSSSPQPK	/	IISNASCTTNCLAPLAK	92	94
ALPNNTSSSPQPK	/	VPTANVSVVDLTCR	97	97
ELNEALELK	/	ELNEALELK	88	90
ELNEALELK	/	IISNASCTTNCLAPLAK	95	96
ELNEALELK	/	VPTANVSVVDLTCR	98	98

Supplementary Figure 5. Second set of RNAi-mediated TP53 depletions was quantified by Selected Reaction Monitoring (SRM) mass spectrometry. SIL peptides ALPNNTSSSPQP[Lys(13C6; 15N2)] and ELNEALEL[Lys(13C6; 15N2)] were added to the WCE for normalization. The chromatograms show sets of transitions for specific peptides derived from target protein TP53 (**a**) or loading control GAPDH (**b**) in human cell RNAi depletion experiments. Samples were treated with a control RNAi and analyzed in duplicate (control 1 and control 2) or with a *TP53*-specific siRNA prior to SRM analysis. Specific peptides detected and quantified from TP53 were ALPNNTSSSPQPK and ELNEALELK, and from GAPDH VPTANVSVVDLTCR and IISNASCTTNCLAPLAK. Panel (**c**) shows quantitation of depletion determined from SRM data, as peak intensities from TP53 peptides ALPNNTSSSPQPK and ELNEALELK were normalized against corresponding SIL peptides, and two GAPDH peptides IISNASCTTNCLAPLAK and VPTANVSVVDLTCR against two analytical replicates of control sample.

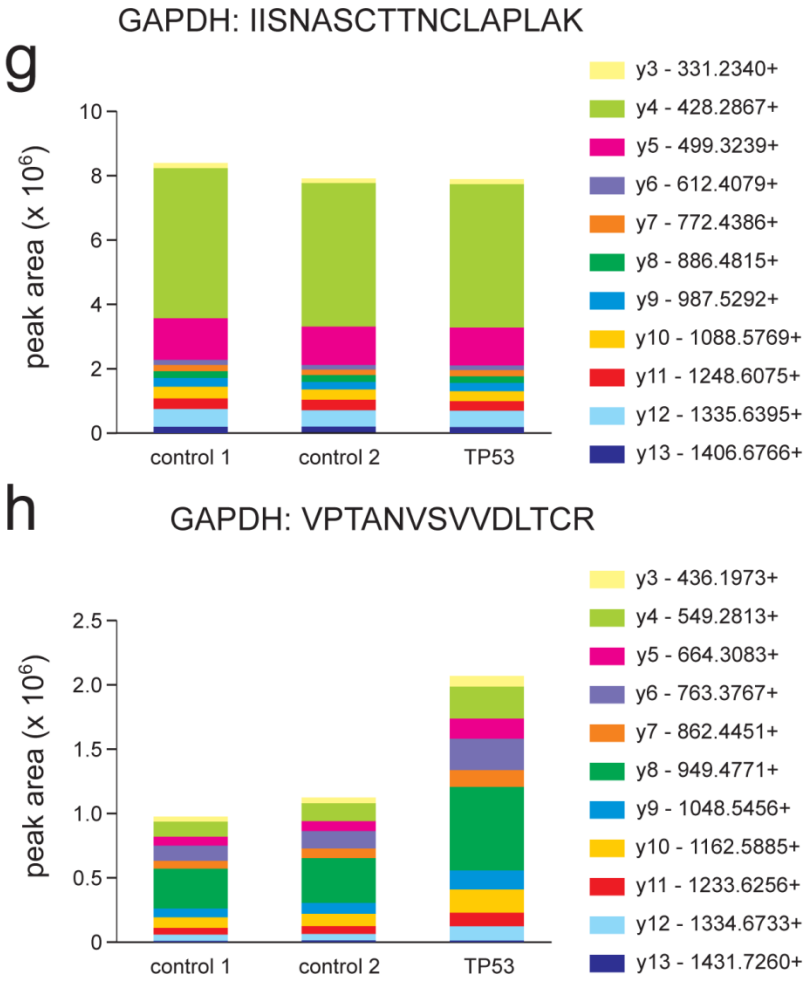
Supplementary figure 6



Supplementary figure 6

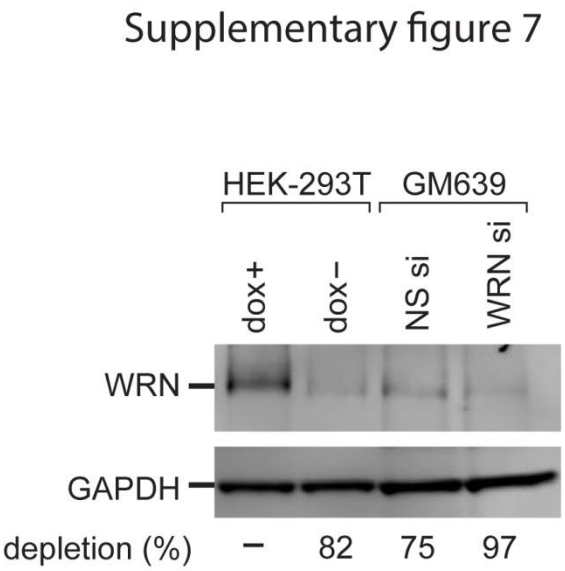


Supplementary figure 6



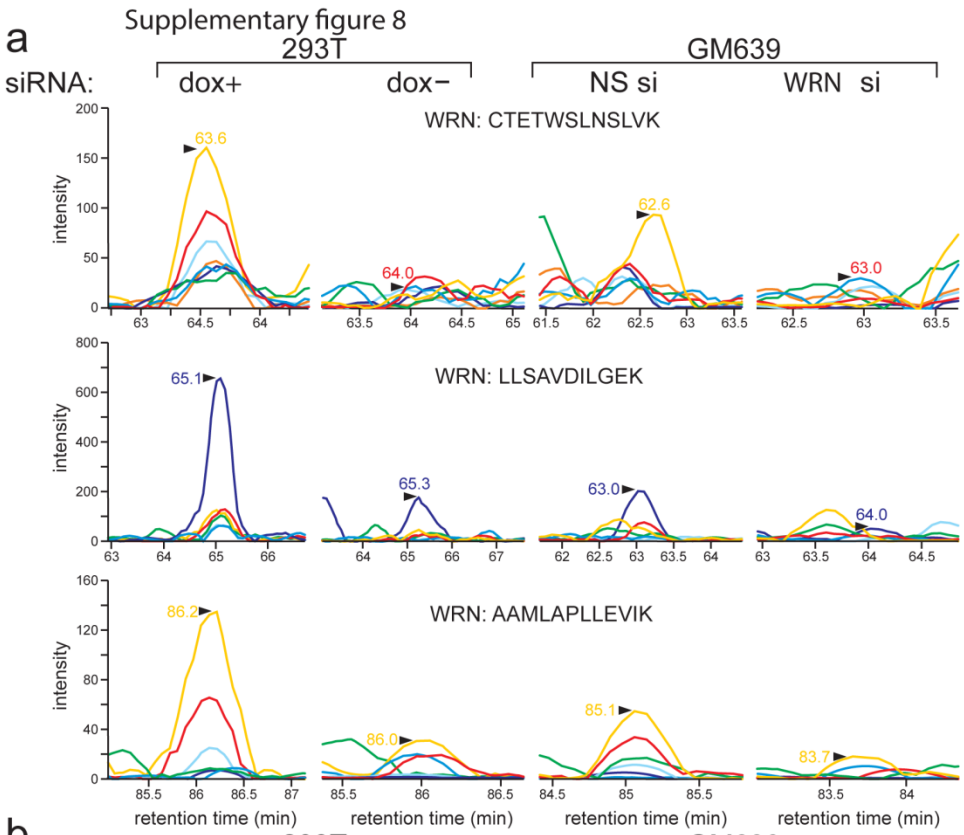
Supplementary Figure 6. Target protein-specific ion ratios are maintained in samples having different protein abundances in samples where SIL were added. Two analytical replicates of control (without knockdown) lysate were run, followed by knockdown sample. Signal from spiked-in heavy peptides remained relatively even in samples with and without knockdown of endogenous protein, as detected for both analyzed TP53 peptides, ALPNNSSSPQPK (**a**) and ELNEALELK (**d**). Absolute (**b**) and normalized (**c**) ion ratios from TP53 peptide

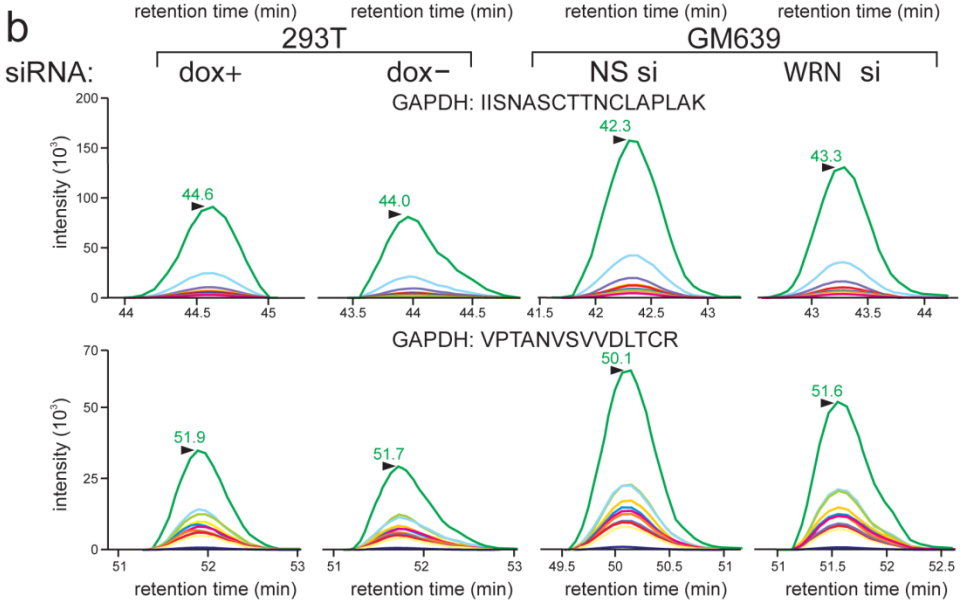
ALPNNSSSPQPK are shown for samples having substantially different amounts of endogenous TP53 protein. Panels (**e**) and (**f**) show ion ratios for TP53 peptide ELNEALELK, (**g**) and (**h**) show ion ratios for GAPDH peptides IISNASCTTNCLAPLAK and VPTANVSVVDLTCR, respectively.



Supplementary Figure 7. Western blot verification of WRN protein induction and depletion from human cells. Sample nomenclature is explained in legend for **Supplementary Figure 8**.

Supplementary figure 8





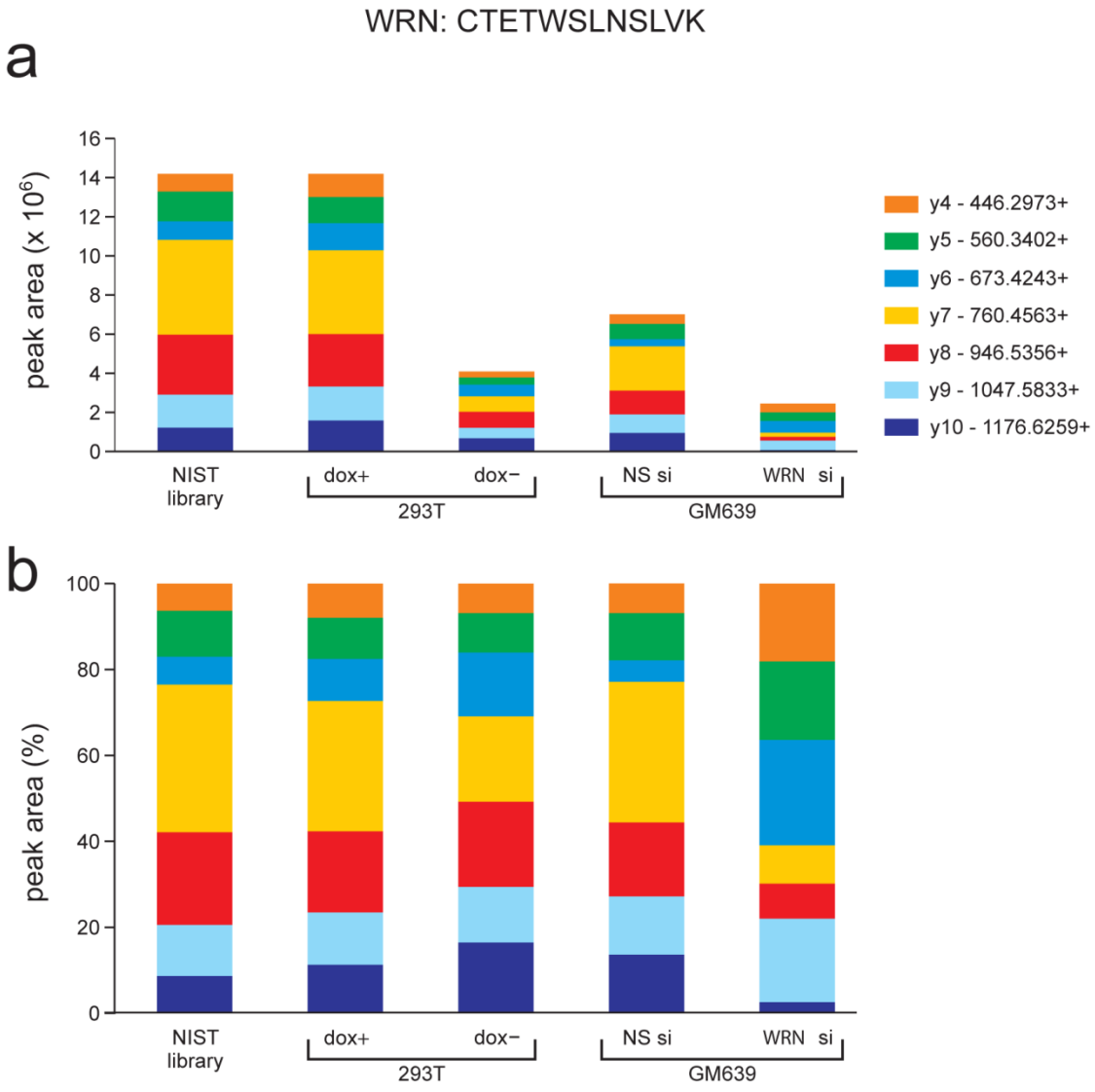
c

WRN peptide	/	control peptide	WRN depletion (%) normalized to dox+ dox−	NS si	WRN si
CTETWSLNSLVK	/	IISNASCTTNCLAPLAK	74.1	78.1	90.5
CTETWSLNSLVK	/	VPTANVSVVDLTCR	69.7	69.2	87.3
LLSAVDILGEK	/	IISNASCTTNCLAPLAK	72.2	84.2	95.5
LLSAVDILGEK	/	VPTANVSVVDLTCR	67.4	77.8	94.0
AAMLAPLLEVIK	/	IISNASCTTNCLAPLAK	83.4	84.8	94.5
AAMLAPLLEVIK	/	VPTANVSVVDLTCR	80.5	78.6	92.6

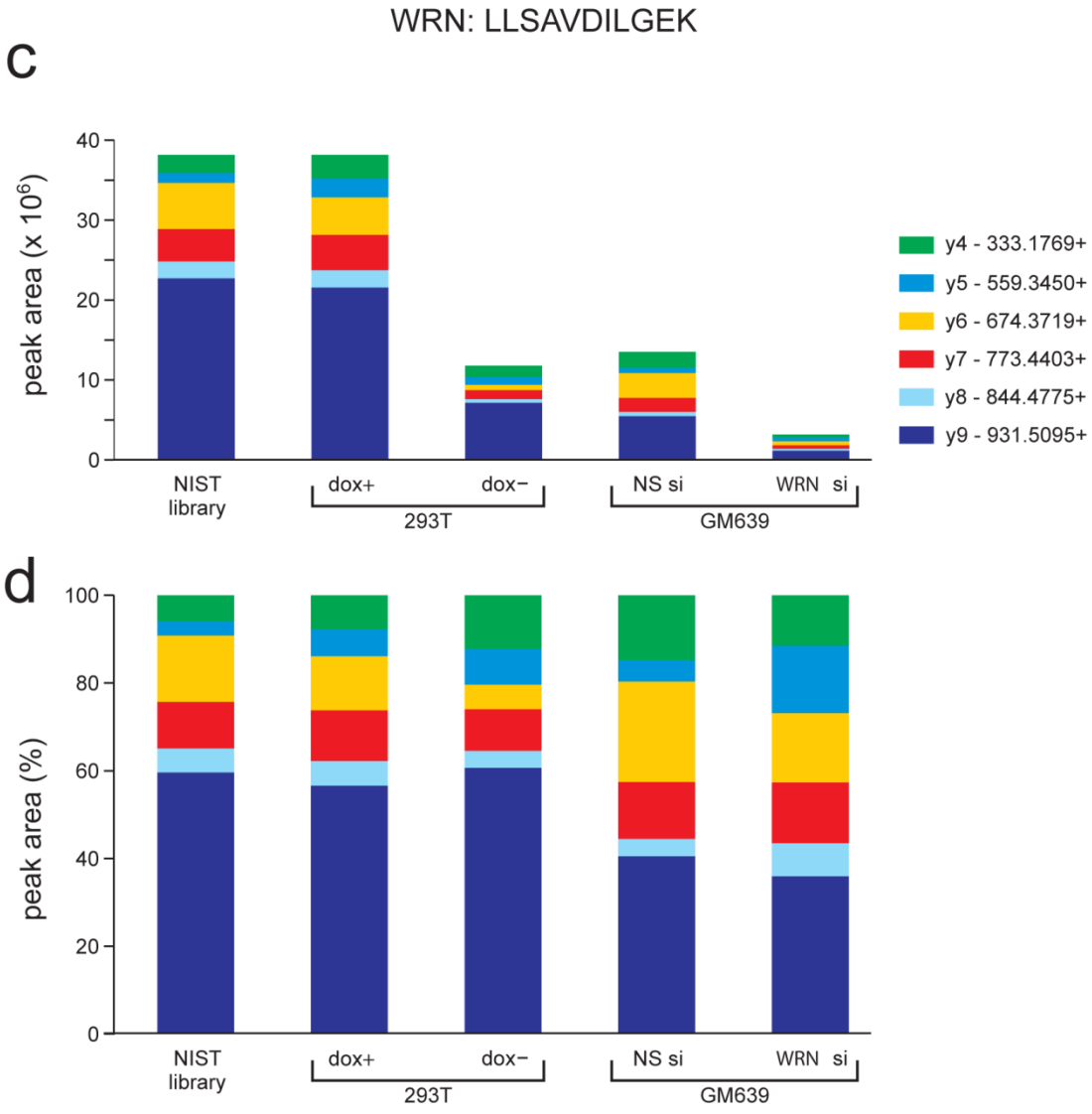
	dot products between experimental data and NIST spectral library			
	dox+	dox−	NS si	WRN si
CTETWSLNSLVK	1.00	0.97	0.99	0.88
LLSAVDILGEK	1.00	0.98	0.97	0.94
AAMLAPLLEVIK	0.99	0.88	0.95	0.85
IISNASCTTNCLAPLAK	0.91	0.91	0.91	0.91
VPTANVSVVDLTCR	0.90	0.90	0.90	0.90

Supplementary Figure 8. RNAi-mediated WRN protein depletion quantified by SRM. Specific peptides detected and quantified from WRN were CTETWSLNSLVK, LLSAVDILGEK, and AAMLAPLLEVIK. Their signal intensity was normalized against GAPDH peptides VPTANVSVVDLTCR and IISNASCTTNCLAPLAK. The chromatograms show sets of transitions for three peptides derived from target protein WRN (**a**) or loading control GAPDH (**b**) in human cell induction and RNAi depletion experiments. Tet-inducible Flp-In T-REx-293 cells were treated with doxycycline to induce increased expression of WRN in "dox+" samples, and were left uninduced for measurement of baseline expression in "dox-" samples. Human GM639 cells were treated with nonspecific RNAi ("NS si") or a *WRN*-specific siRNA ("*WRN* si") prior to SRM analysis. (**c**) TP53 depletion was quantified from individual peak area ratios determined for all measured peptides and normalized against an internal standard GAPDH as described in Methods. Dot products were calculated by Skyline to characterize the agreement between ion fragment ratios in our experimental data and NIST spectral library for each measured peptide.

Supplementary figure 9



Supplementary figure 9



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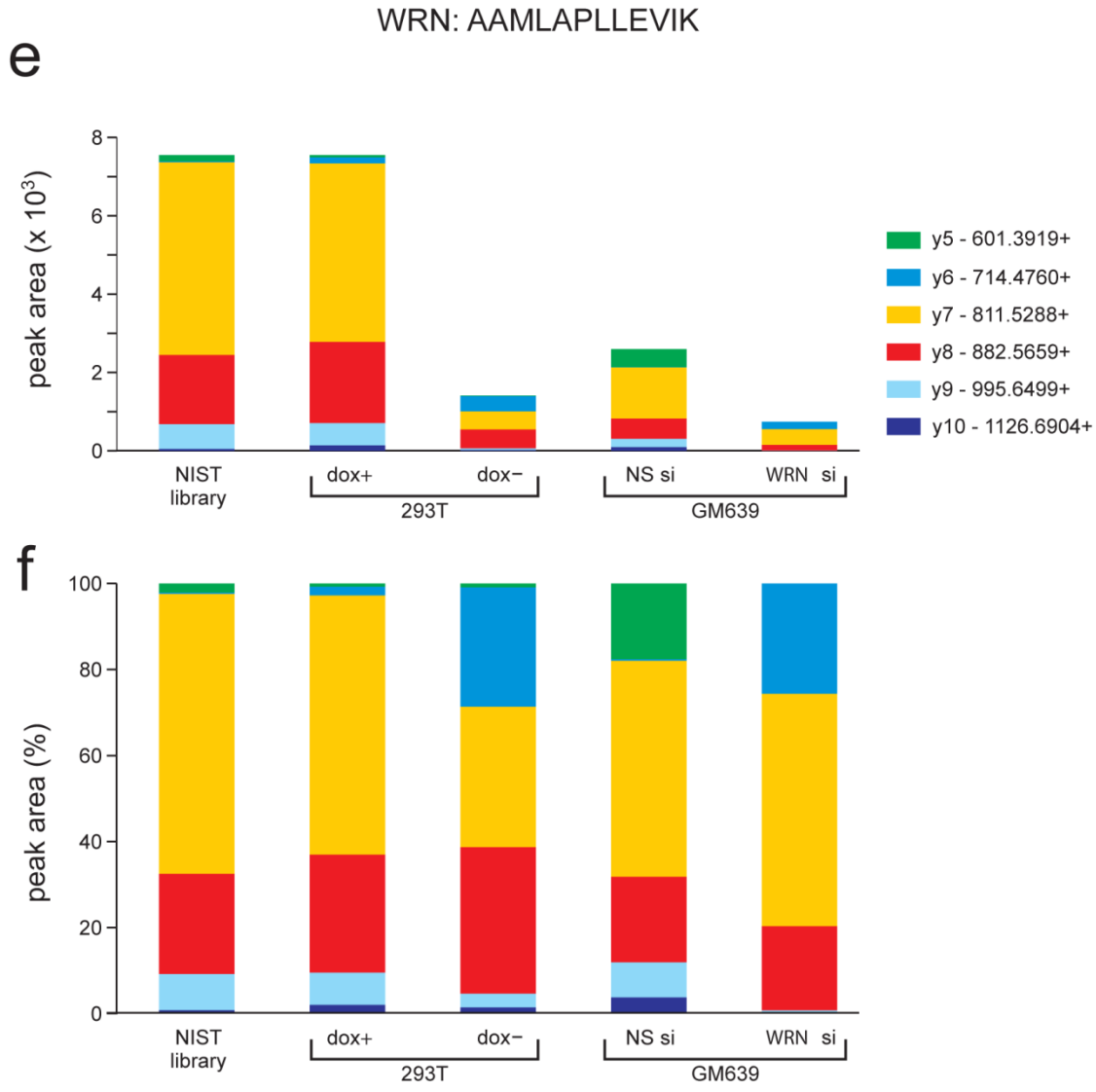

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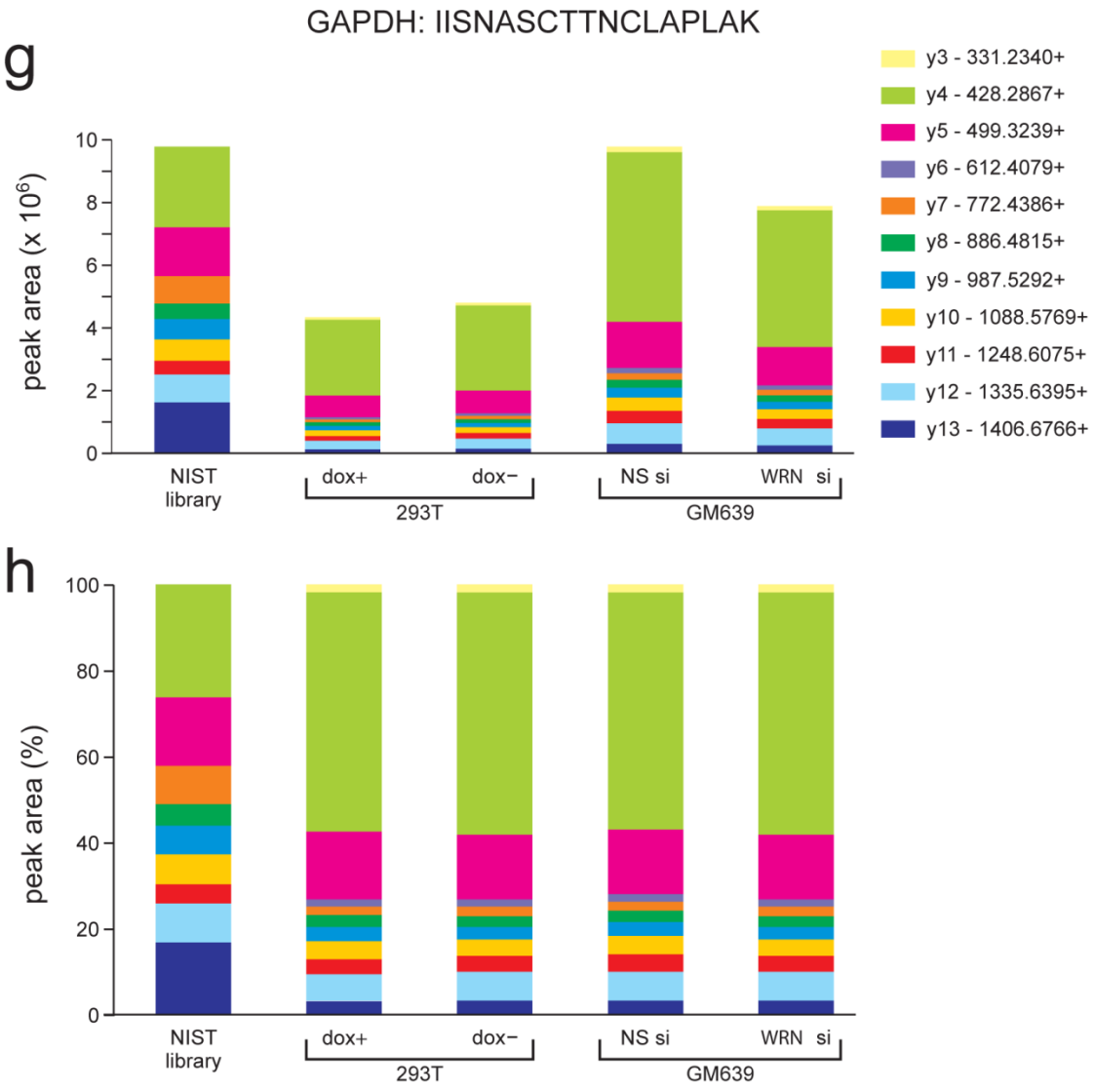

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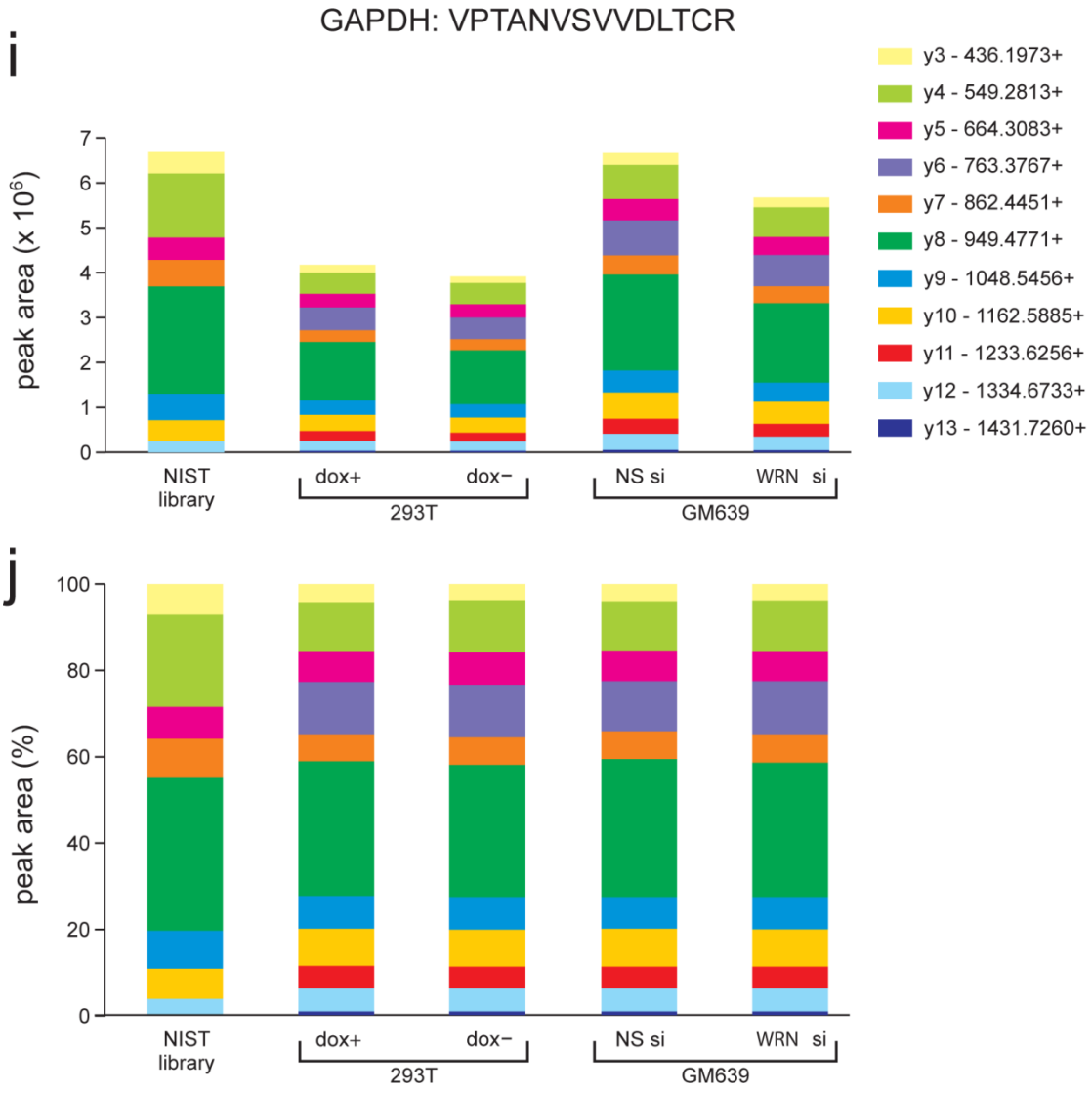
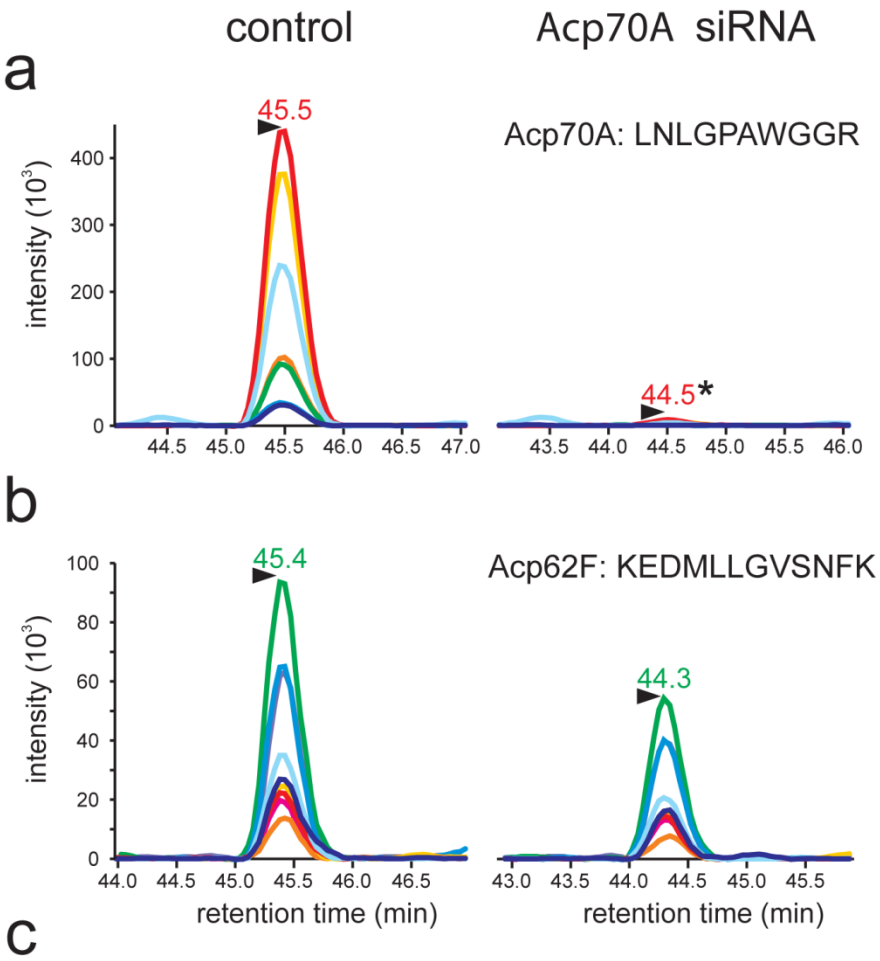


Supplementary Figure 9. Target protein-specific ion ratios are verified against NIST spectral library, and maintained in samples expressing different amounts of WRN protein. Sample nomenclature is explained in legend for **Supplementary Figure 8**.

Absolute and normalized ion ratios are demonstrated for WRN peptides CTETWSLNSLVK, LLSAVDILGEK, and AAMLAPLLEVIK in panels (**a – f**), as well as GAPDH peptides VPTANVSVVDLTCR and IISNASCTTNCLAPLAK in panels (**g – j**).

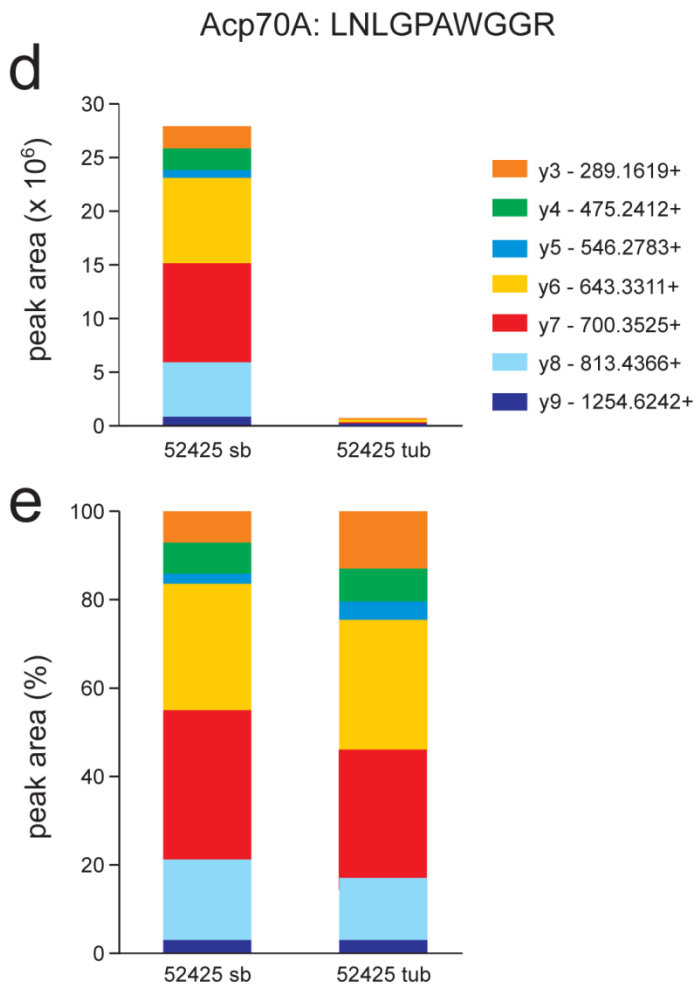
Supplementary figure 10



c

Acp70A peptide	/	Acp62F peptide	Acp70A depletion (%)
LNLGPAWGGR	/	KEDMLLGVSNFK	>99*

Supplementary figure 10



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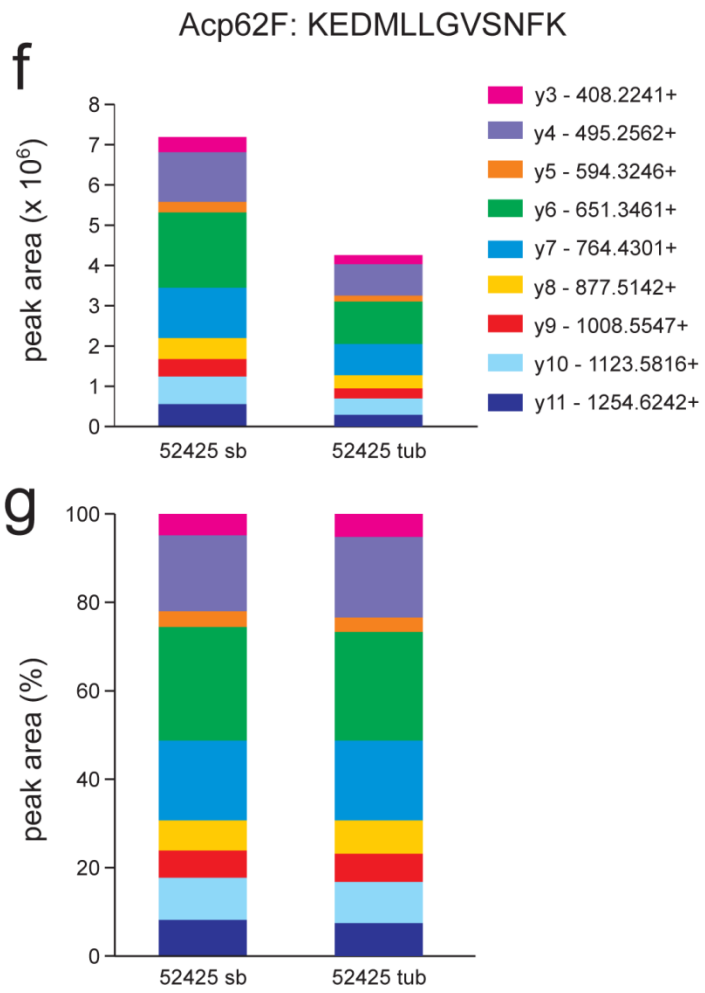
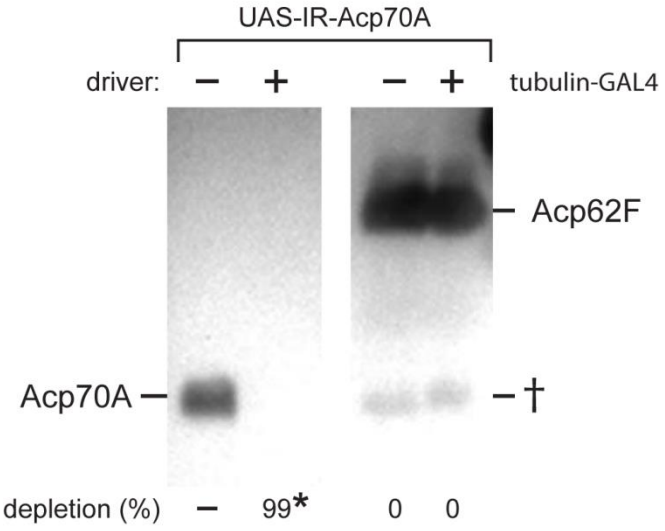


Supplementary Figure 10. Ion chromatograms depicting RNAi-mediated depletion of accessory gland seminal fluid protein Acp70A from whole *Drosophila*. (**a**) SRM ion chromatograms for Acp70A peptide LNLGPAWGGR at *m/z* 520.8. (**b**) corresponding ion chromatograms for control protein Acp62F peptide KEDMLLGVSNFK at *m/z* 690.3. (**c**) quantitation of percent depletion of RNAi target protein Acp70A versus Acp62F control based on SRM results. Peptide intensities were normalized against internal standard Acp62F within each sample, then compared

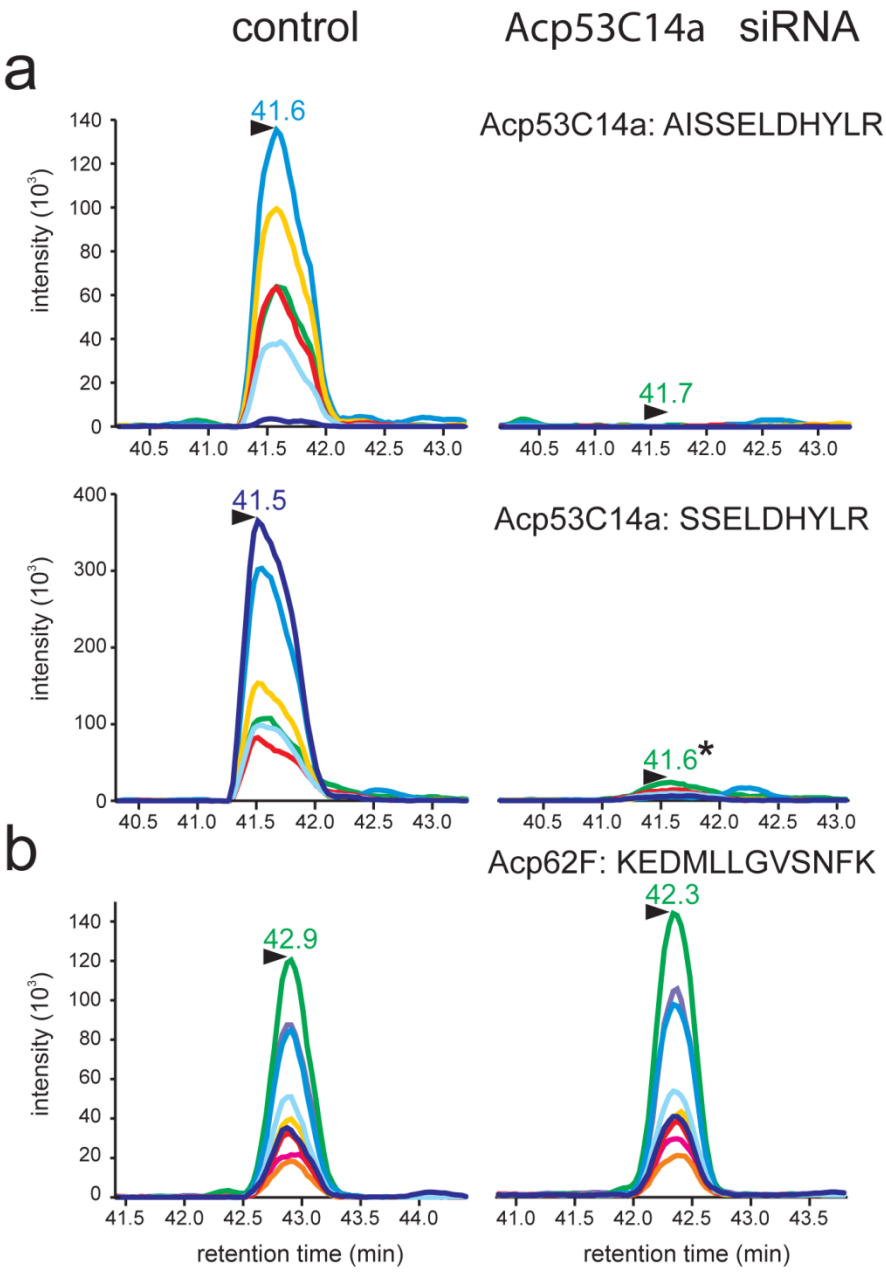
between depleted and control samples to quantify Acp70A depletion. Residual signal in ion chromatograms for peptide LNLGPAWGGR (*) is derived from contaminants that are not Acp70A-derived (see text), and thus have been excluded from quantitation. Panels (**d**) and (**e**) show absolute and normalized ion ratios for Acp70A peptide LNLGPAWGGR, and (**f**) and (**g**) show absolute and normalized ion ratios for Acp62F peptide KEDMLLGVSNFK.

Supplementary figure 11



Supplementary Figure 11. Western blot analysis of RNAi-mediated depletion of accessory gland seminal fluid protein Acp70A from whole *Drosophila.* Left panel: Acp70A was detected in control (*tubulin-GAL4* driver-minus (−) flies but not in RNAi expressing (+) flies (see Methods). Right panel: Internal control protein Acp62F, in contrast, was detected in both samples. A cross-reacting band detected by the anti-Acp62F antibody is denoted by a dagger (†). The asterisk (*) indicates percent Acp70A-specific depletion normalized to Acp62F in the same samples analyzed in **Supplementary Figure 10**.

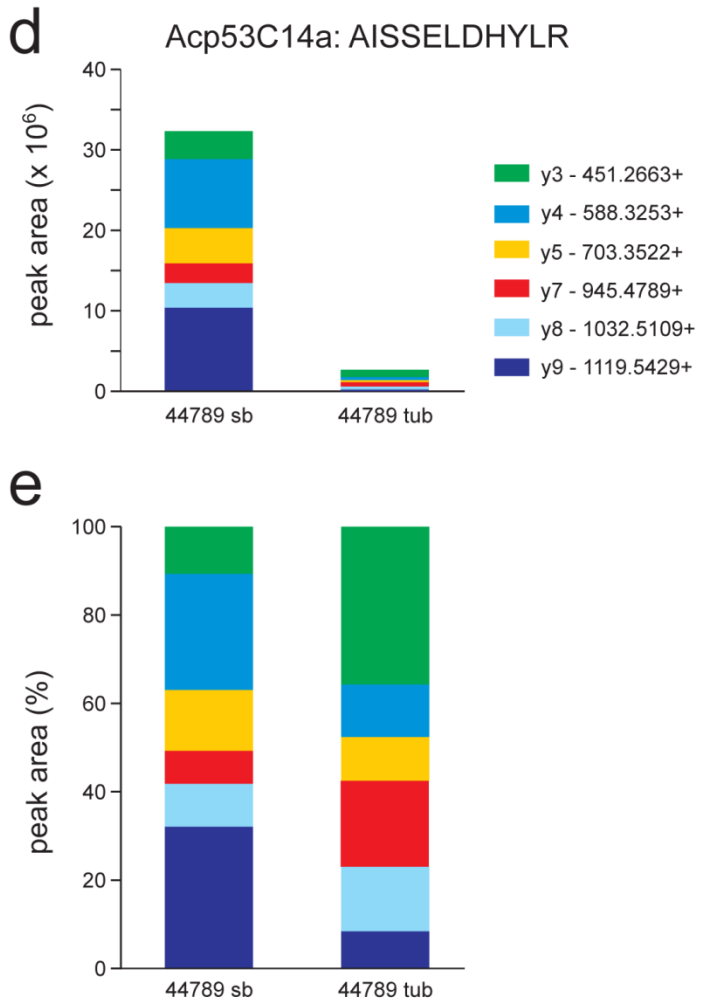
Supplementary figure 12



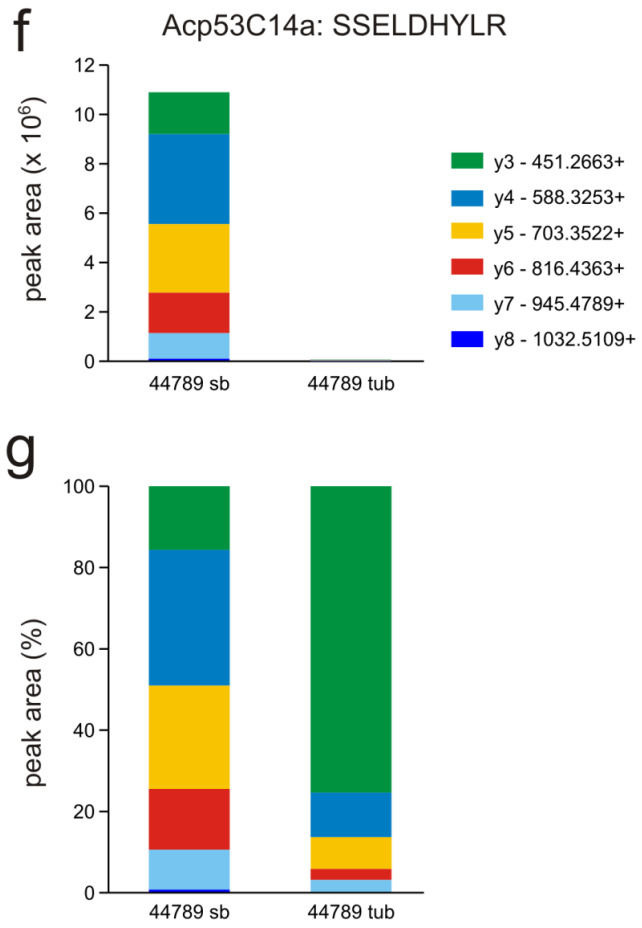
c

Acp53C14a peptide	/	Acp62F peptide	Acp53C14a depletion (%)
AISSELDHYLR	/	KEDMLLGVSNFK	93
SSELDHYLR	/	KEDMLLGVSNFK	>99*

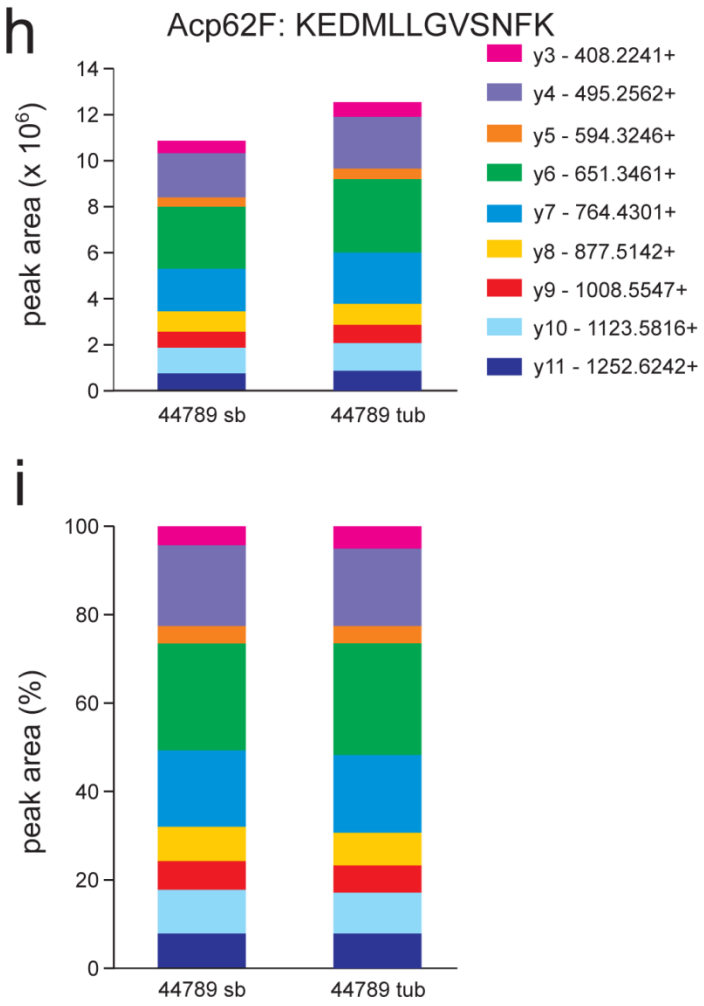
Supplementary figure 12



Supplementary figure 12



Supplementary figure 12



Supplementary Figure 12. Ion chromatograms depicting RNAi-mediated depletion of accessory gland seminal fluid protein Acp53C14a from whole *Drosophila*. SRM ion chromatograms for (**a**) Acp53C14a peptides AISSELDHYLR at *m/z* 652.3 and SSELDHYLR at *m/z* 560.3 in flies expressing no (left column) or an *Acp53C14a*-specific siRNAi (right column), or for (**b**) control protein Acp62F peptide KEDMLLGVSNFK at *m/z* 690.3. (**c**) quantitation of percent depletion of

RNAi target protein Acp53C14a target peptide versus Acp62F control peptide. Peptide intensities were again normalized against internal standard Acp62F, then compared between depleted and control samples to quantify percent depletion of Acp53C14a. Residual signal in ion chromatograms for peptide SSELDHYLR (*) represents contaminants that are not Acp53C14a-derived, and thus have been excluded from quantitation.

Panels (**d**) and (**e**) show absolute and normalized ion ratios for Acp53C14a peptide AISSELDHYLR, (**f**) and (**g**) show absolute and normalized ion ratios for Acp53C14a peptide SSELDHYLR, and panels (**h**) and (**i**) show absolute and normalized ion ratios for Acp62F peptide KEDMLLGVSNFK.