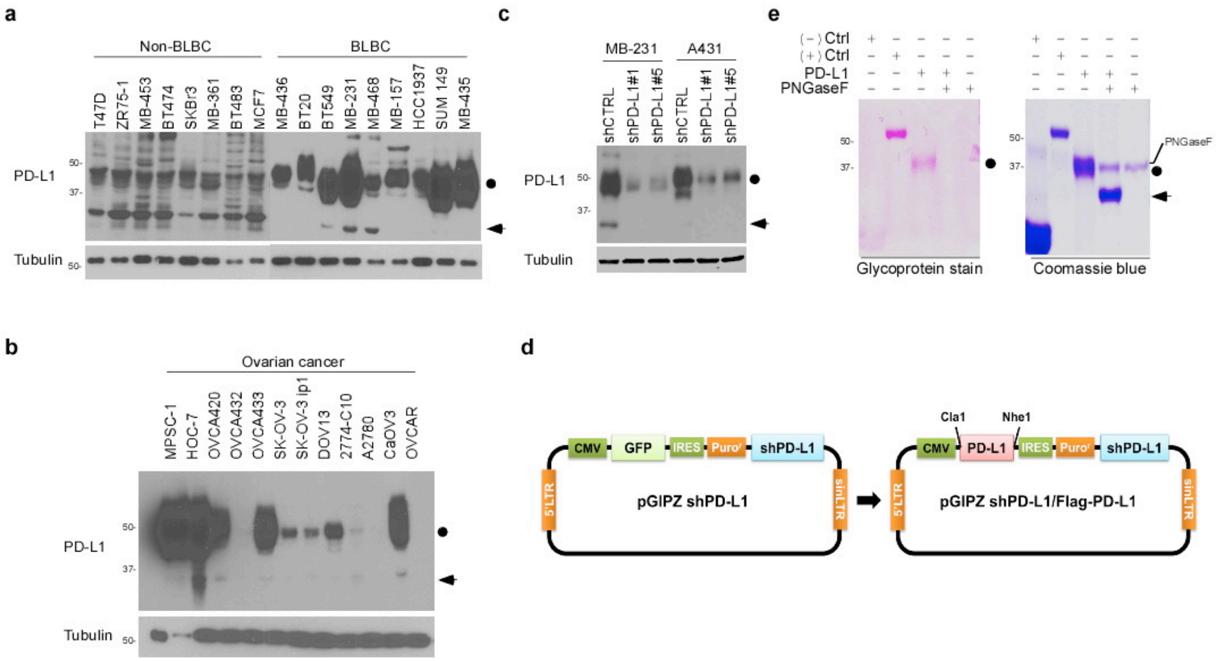
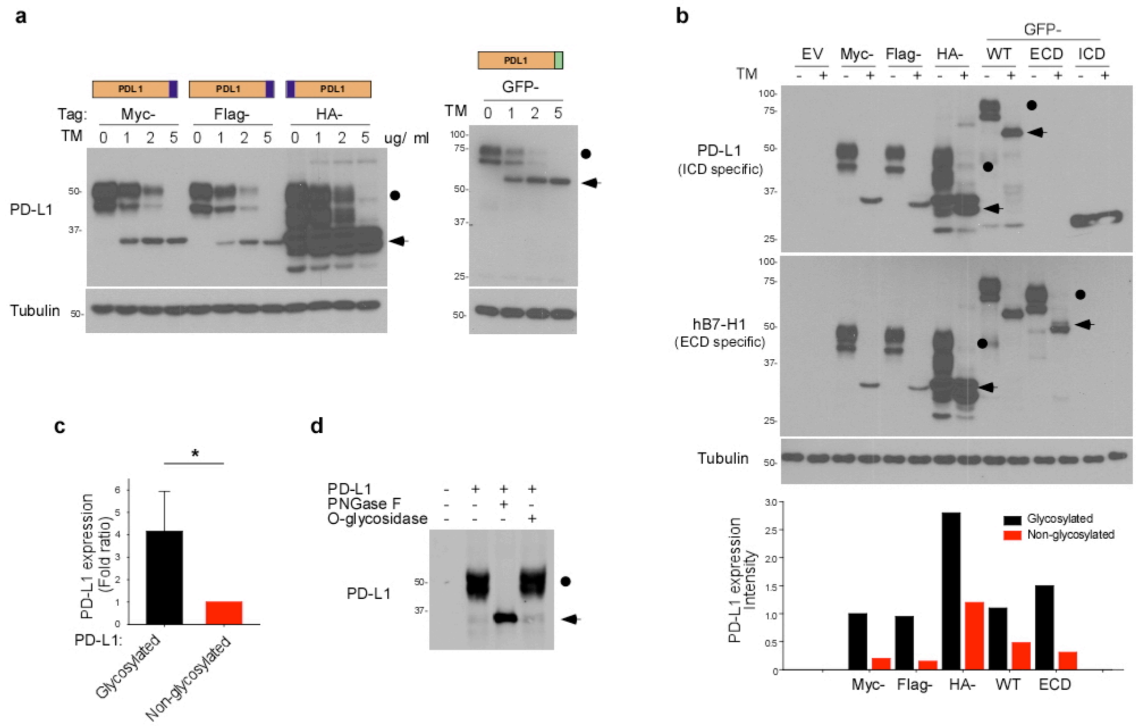
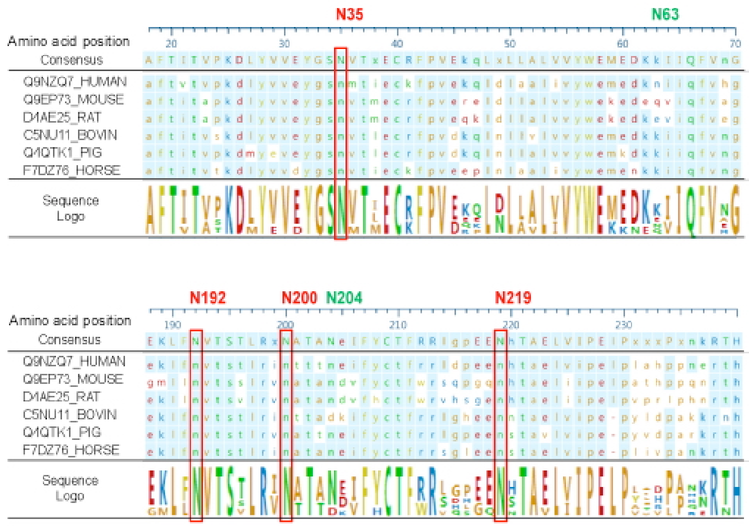


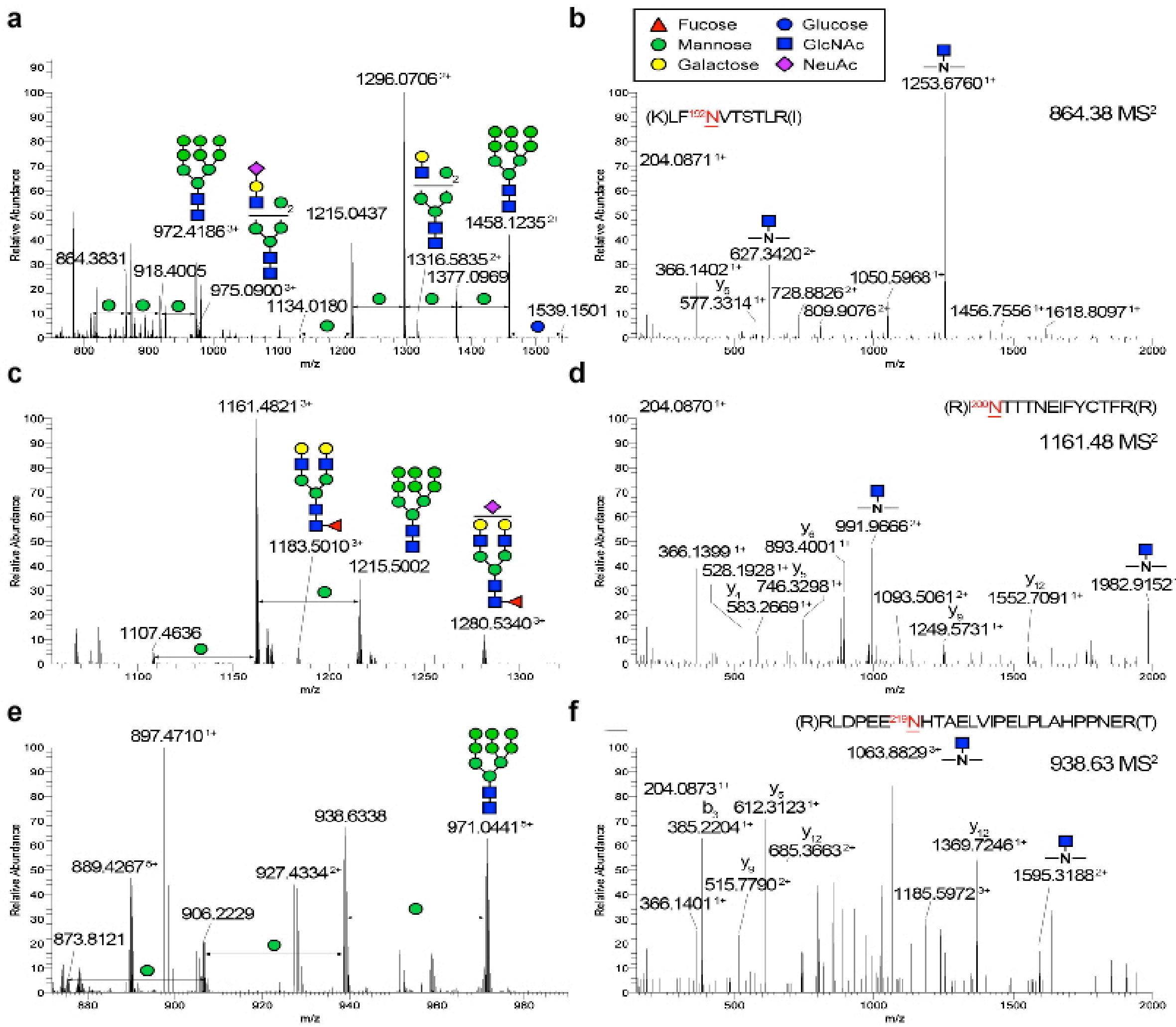
Supplementary Figure 1. PD-L1 is glycosylated in cancer cells. (a) Western blot analysis of PD-L1 in breast cancer cells. **(b)** Western blot analysis of PD-L1 in ovarian cancer cells. **(c)** Western blot analysis of PD-L1 in shCTRL and two independent shPD-L1 stable clones of MDA-MB-231 and A431 cells. shCTRL, control shRNA. **(d)** Dual-expression construct for Flag-PD-L1 and shRNA of PD-L1. **(e)** Glycoprotein staining of purified PD-L1 protein with or without PNGase F treatment. Coomassie blue staining panel represents total amount of PD-L1 protein. The upper bands appear in lane 4 and 5 are from the loading of PNGase F. (–) Ctrl, a control for non-glycoprotein; (+) Ctrl, a control for glycoprotein.



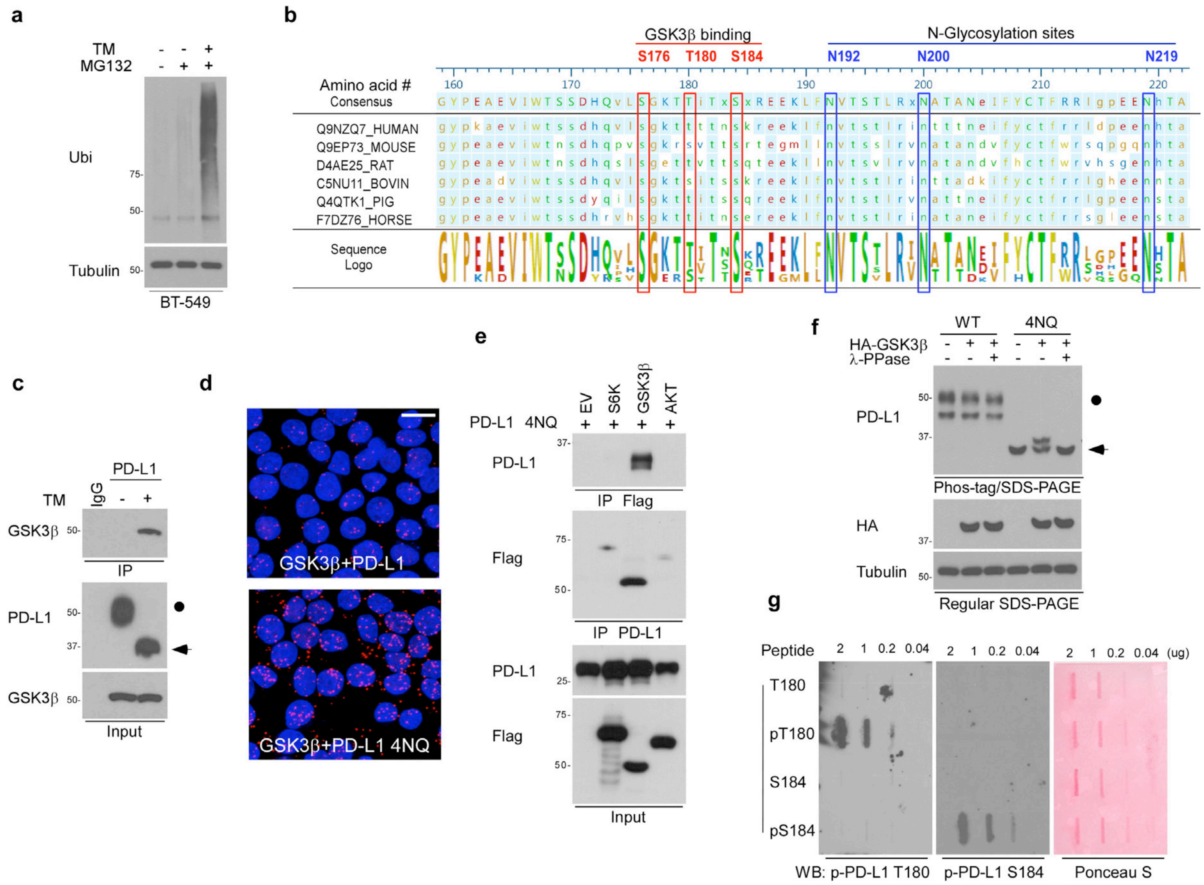
Supplementary Figure 2. Expression of glycosylated and non-glycosylated PD-L1 protein. **(a)** Western blot analysis of PD-L1-Myc, PD-L1-Flag, HA-PD-L1 and PD-L1-GFP proteins in Tunicamycin (TM) treated cells. **(b)** Western blot analysis of PD-L1-Myc, PD-L1-Flag, HA-PD-L1, PD-L1-GFP WT, ECD, and ICD proteins in TM treated cells. **(c)** The mean of the intensity of glycosylated (black bar) or non-glycosylated PD-L1 (red bar) protein obtained from the bottom of **(b)**. **(d)** Glycosylation pattern of PD-L1 protein in PD-L1 expressing cells. Cell lysates were treated with PNGase F or O-glycosidase and analyzed by Western blot. ECD, extracellular domain; ICD, intracellular domain; Black circle, glycosylated PD-L1; arrowhead, non-glycosylated PD-L1; *P < 0.05 by Student's *t* test. All error bars are expressed as mean ±SD of 3 independent experiments.



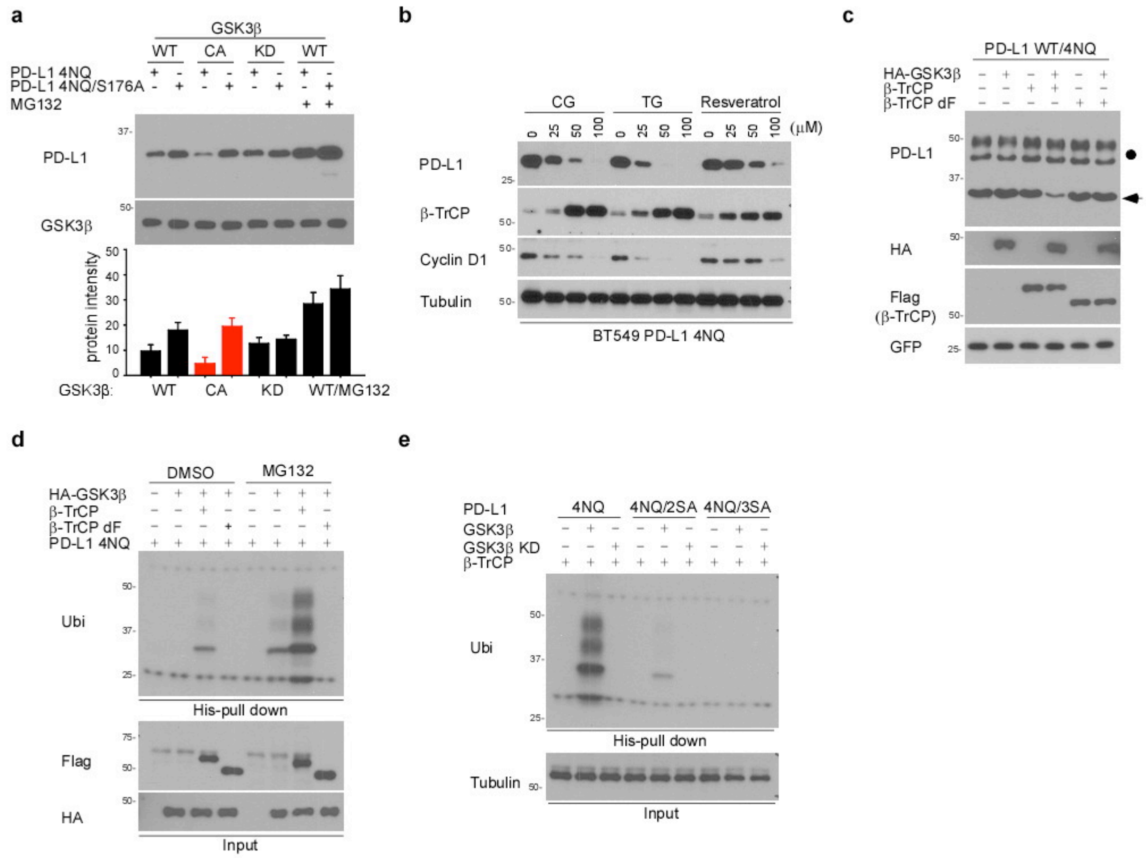
Supplementary Figure 3. N-glycosylation sites of PD-L1 protein. Sequence alignment of the PD-L1 amino acid sequences from different species. Four NXT motifs, N35, N192, N200, and N219 are highlighted in red and two non-NXT motifs, N63 and N204, in green. Red box, conserved NXT motif.



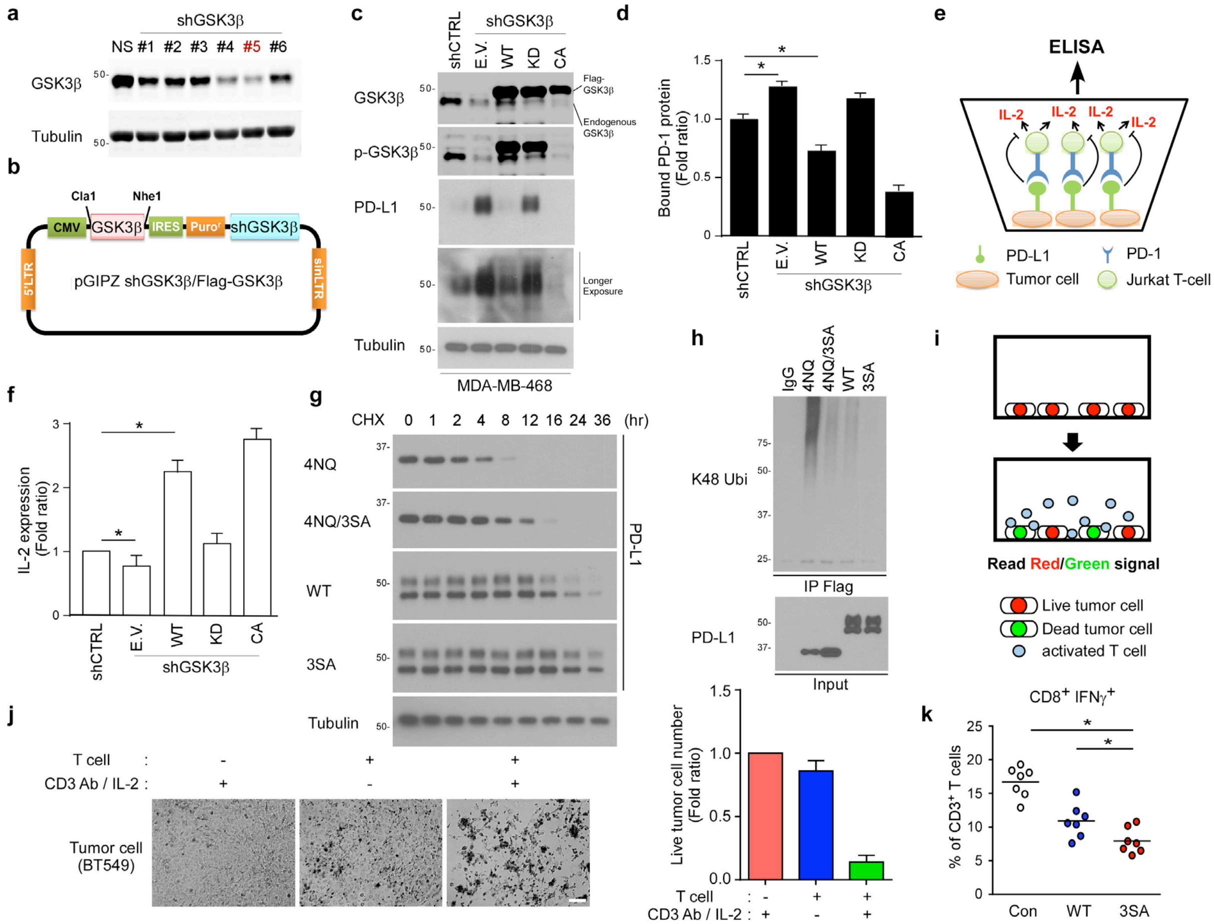
Supplementary Figure 4. N-glycosylation sites of PD-L1 protein. (a)-(f) LC-MS/MS-based identification of *N*-glycopeptides corresponding to each of the four *N*-glycosylation sites, N35, N192, N200, and N219 of PD-L1 (from BT549 cells). The LC-MS profiles **(a, c, and e)** are shown as spectra averaged over a period of elution time (as labeled in figures) when a representative subset of glycoforms were detected. For each *N*-glycosylation site, one representive HCD MS^2 spectrum **(b, d, and f)** is shown to exemplify its identification based on detection of y1 ion (tryptic peptide backbone carrying the GlcNAc attached to the N-glycosylated Asn), along with the b and y ions defining its peptide sequence. The cartoon symbols used for the glycans (see inset) conform to the standard representation recommended by the Consortium for Functional Glycomics: Additional Hex and HexNAc were tentatively assigned as either lacNAc (Gal-GlcNAc) or lacdiNAc (GalNAc-GlcNAc) extension from the trimannosyl core (Man_3-$GlcNAc_2$), which can be core fucosylated.



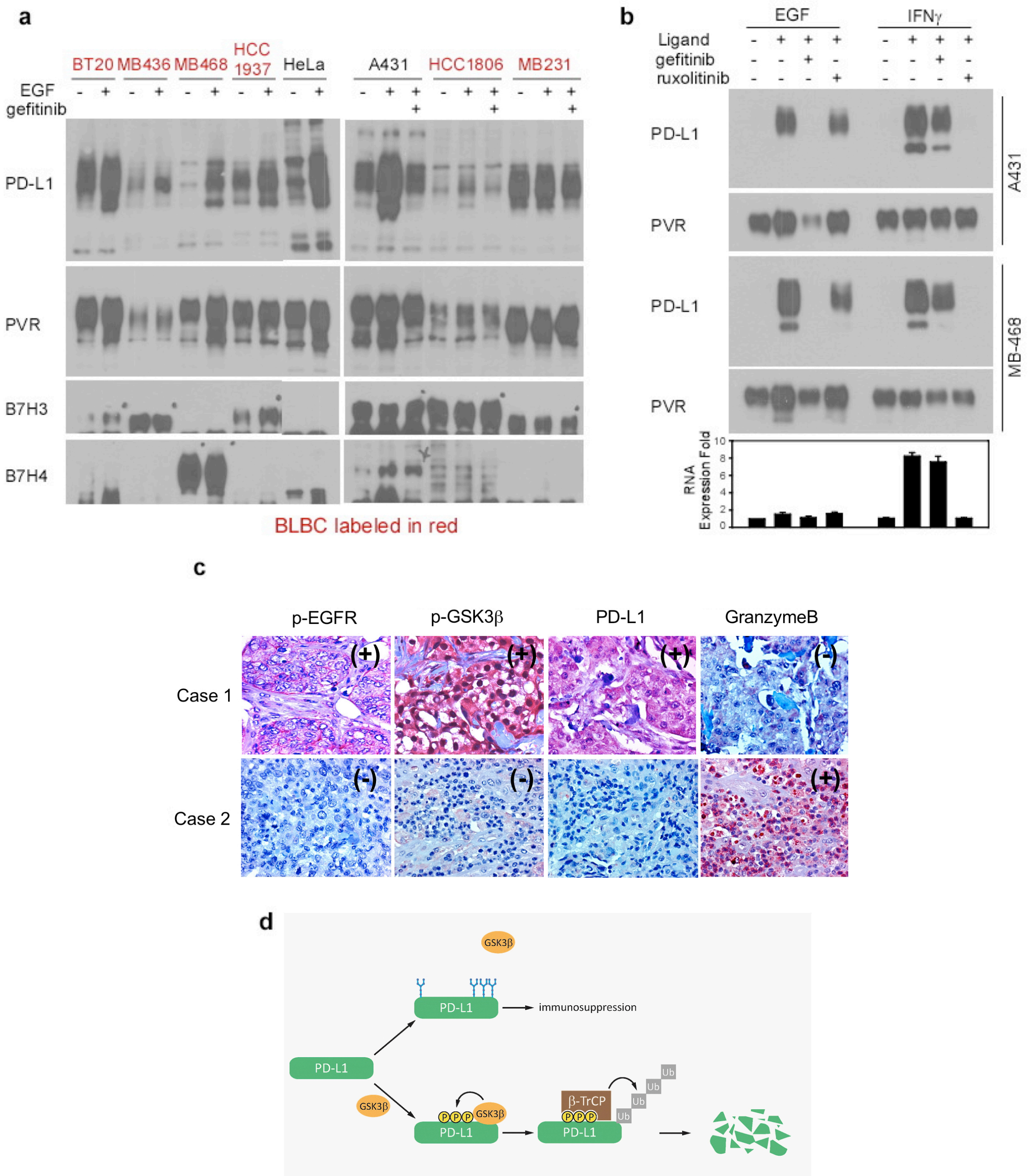
Supplementary Figure 5. GSK3β interacts and phosphorylates PD-L1 (a) Ubiquitination of non-glycosylated PD-L1. Endogenous PD-L1 was immunoprecipitated and subjected to Western blotting with ubiquitin antibody. **(b)** Sequence alignment of the PD-L1 amino acid sequences from different species. Three NXT motifs, N192, N200, and N219 are highlighted in blue and GSK3β phosphorylation motif, S176, T180 and S184, in red. **(c)** Co-immunoprecipitation measuring the interaction of GSK3β and PD-L1. MDA-MB-231 cells were pretreated with 5 μg/ml Tunicamycin (TM) or DMSO for overnight. Endogenous PD-L1 was immunoprecipitated with PD-L1 antibody and subjected to Western blotting with GSK3β antibody. **(d)** PD-L1 WT or 4NQ expressing BT549 cells were immunostained with GSK3β and PD-L1 antibodies and assessed using Duolink® II assay. Red foci indicate interactions between GSK3β and PD-L1 WT or 4NQ proteins. Scale bar, 20 μm. **(e)** The binding affinity between PD-L1 4NQ and GSK3β. HEK 293 cells were transient transfected with indicated plasmid and then subject to immunoprecipitation followed by Western blotting. **(f)** Analysis of PD-L1 phosphorylation using Phos-tag/SDS–polyacrylamide gel (PAGE). HEK 293T cells transfected with PD-L1 WT or PD-L1 4NQ together with HA-GSK3β CA were separated by Phos-tag/SDS–PAGE. Undenatured samples were subject to λ-PPase treatment for 1 hr before resolving on the Phos-tag/SDS–PAGE. **(g)** Characterization of phospho-PD-L1 antibodies. Black circle, glycosylated PD-L1; arrowhead, non-glycosylated PD-L1.



Supplementary Figure 6. GSK3β induces β-TrCP-mediated PD-L1 ubiquitination and degradation. (a) Western blot analysis of PD-L1 4NQ expression. Various GSK3β were transfected together with PD-L1 4NQ to test their ability degrading PD-L1 4NQ. **(b)** BT549 PD-L1 4NQ cells were treated with CG, TG and resveratrol with the indicated concentration for 48 h. Protein expression was analyzed by Western blotting. **(c)** BT-549-PD-L1 4NQ cells were transfected with plasmids expressing HA-GSK3β, Flag-β-TrCP or Flag-β-TrCP dF and analyzed by Western blot. **(d, e)** Ubiquitination of PD-L1 requires GSK3β phosphorylation. GSK3β, PD-L1 4NQ and β-TrCP were transient transfected in HEK 293T cells. Ubiquitinated PD-L1 was pull down by His-tagged Ubiquitin and analyzed by Western blot. Black circle, glycosylated PD-L1; arrowhead, non-glycosylated PD-L1.

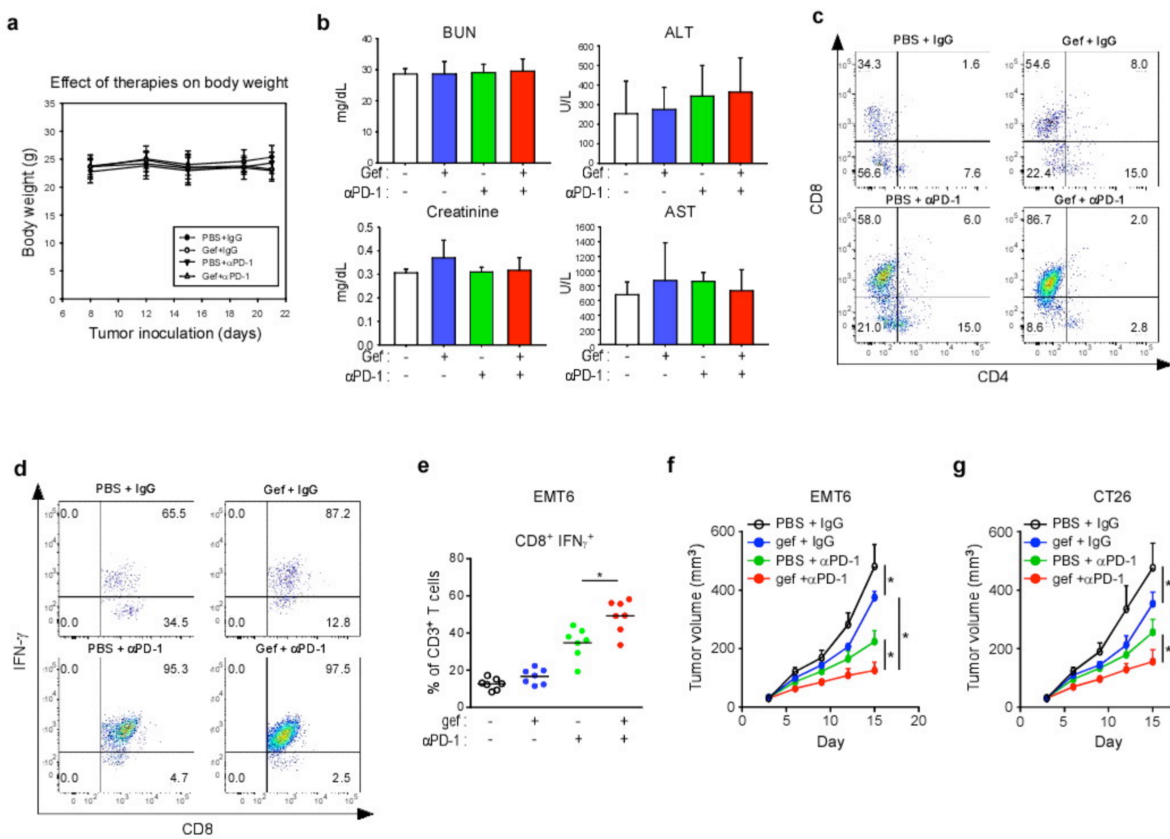


Supplementary Figure 7. (a) Characterization of GSK3β shRNA clones. **(b)** Vector design of GSK3b knockdown and reconstitution. **(c)** Western blot analysis of stable clones with GSK3β variants. **(d)** PD-1 binding assay of GSK3β stable clones from **(c)**. **(e)** Schematic diagram of IL-2 ELISA. PD-L1 stable clones were co-cultured with PD-1 overexpressed Jurkat T cells. IL-2 expression from Jurkat T cells was measured by ELISA. **(f)** Jurkat T cells IL2 expression of GSK3β stable clones from **(c)**. **(g, h)** Western blot analysis of PD-L1 4NQ, 4NQ/3SA, WT, and 3SA protein stability. Cells were treated with or without 20 mM CHX as indicated intervals shown in **(g)**. Protein ubiquitination is shown in **(h). (i)** Schematic diagram of T cell-mediated tumor cell killing assay. Nuclear restricted RFP expressing tumor cells and activated T cell are co-cultured in presence of Caspase 3/7 substrate. T cells were activated with anti-CD3 antibody (100 ng/ml) and IL-2 (10 ng/ml). At 96 hr, RFP and green fluorescent (NucView^{TM} 488 Caspase 3/7 substrate) signal were measured. Green fluorescent cell was counted as dead cell. **(j)** Representative phase images (10x) of BT549 cells and/or activated T cells co-cultures at 96 hr. T cells were activated with anti-CD3 antibody (100 ng/ml) and IL-2 (10 ng/ml). The quantitative ratio of live cells is shown in bar graph (right). Scale bar, 100 µm. **(k)** Intracellular cytokine stain of IFNγ in CD8$^+$ CD3$^+$ T cell populations from the isolated TIL. Con, vector control expressing cells; WT, PD-L1 WT expressing cells; 3SA, PD-L1 3SA expressing cells. * $P < 0.05$ by Student's *t* test. All error bars are expressed as mean ±SD of 3 independent experiments.



Supplementary Figure 8. EGF induces PD-L1 glycosylation. (a) Western blot analysis of PD-L1 glycosylation in various cells. Cells were serum starvation for overnight and then treated with 25 ng of EGF. **(b)** Western blot analysis of PD-L1 expression upon EGF or IFNγ treatment. The mRNA expression of individual treatment was shown in the bottom. **(c)** Representative images from IHC staining of p-EGFR (phosphorylation of Tyr 1068), p-GSK3β (phosphorylation of Ser

9), PD-L1, and granzyme B in primary breast cancer patients. **(d)** A proposed model of EGF mediated PD-L1 stabilization contributing to T cell immune escape. Black circle, glycosylated PD-L1; arrowhead, non-glycosylated PD-L1. All error bars are expressed as mean ±SD of 3 independent experiments.



Supplementary Figure 9. T cell profile analysis from tumor infiltrated lymphocytes. (a) The effect of treatment on mice body weight. **(b)** Mice liver and kidney functions were measured at the end of the experiments. **(c)** Flow cytometry of CD8 and CD4 markers on $CD3^+$ T cells isolated from tumors of gefitinib and/or anti-PD-1 antibody treated mice. **(d)** Flow cytometry of IFNγ and CD8 markers on $CD8^+$ $CD4^-$ T cells isolated from tumors of gefitinib and/or anti-PD-1 antibody-treated mice. Error bars represent mean ±SD of 3 independent experiments. **(e)** Intracellular cytokine stain of $IFN\gamma^+$ $CD8^+$ in $CD3^+$ T cell populations from the isolated TIL. **(f)** The tumor growth of EMT6 cells in gefitinib and/or anti-PD-1 antibody treated BALB/c mice. **(g)** The tumor growth of CT26 cells in gefitinib and/or anti-PD-1 antibody treated BALB/c mice.

Fig.1a

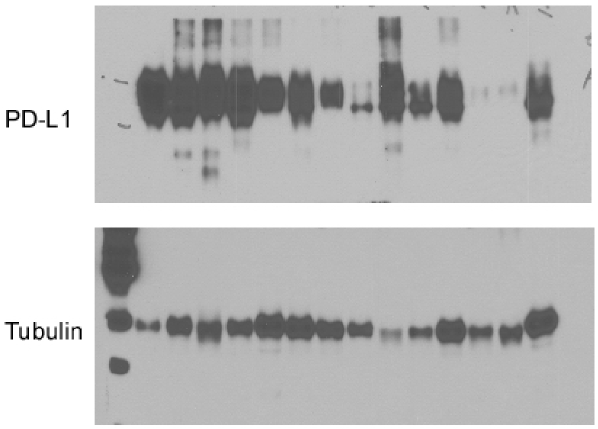


Fig.1h

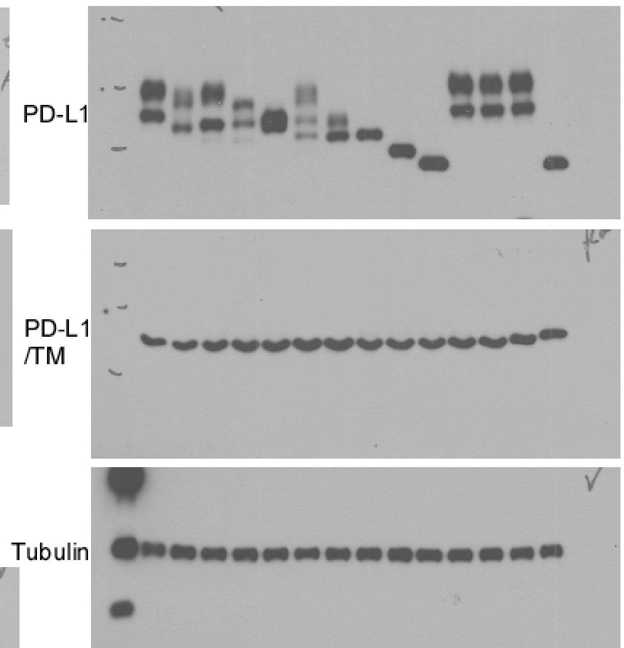


Fig.1b

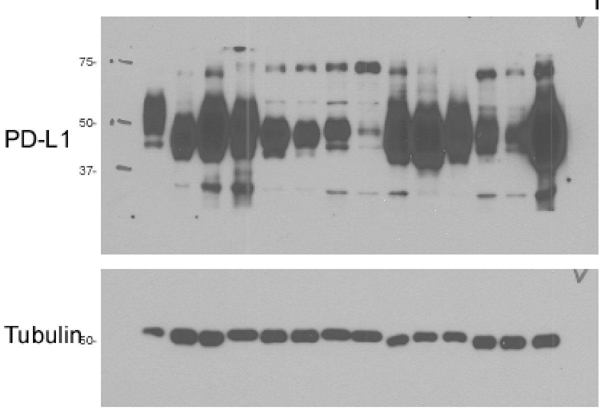


Fig.1e

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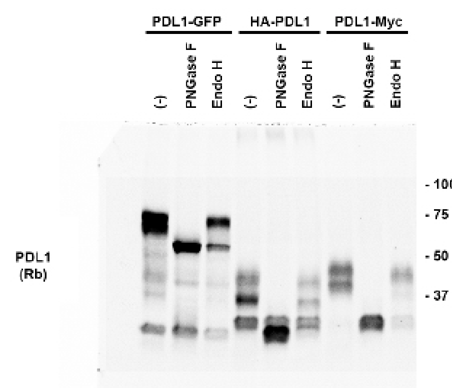


Fig.1c

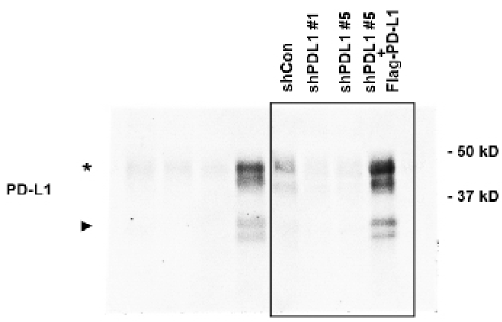
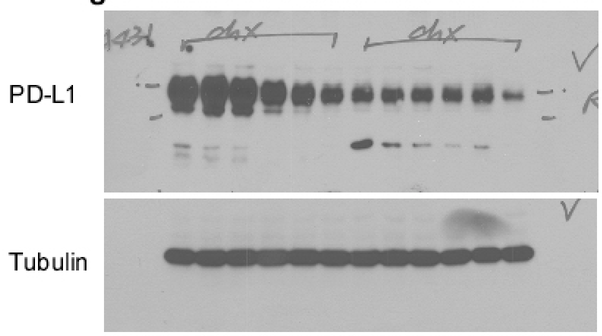


Fig. 2a



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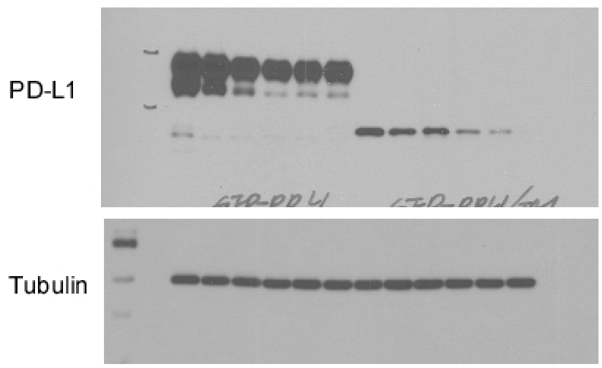

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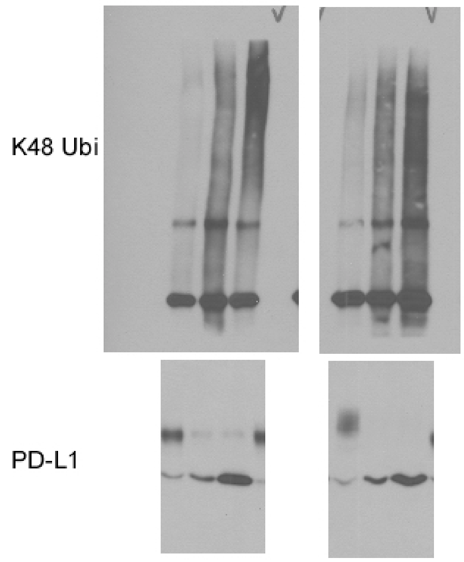

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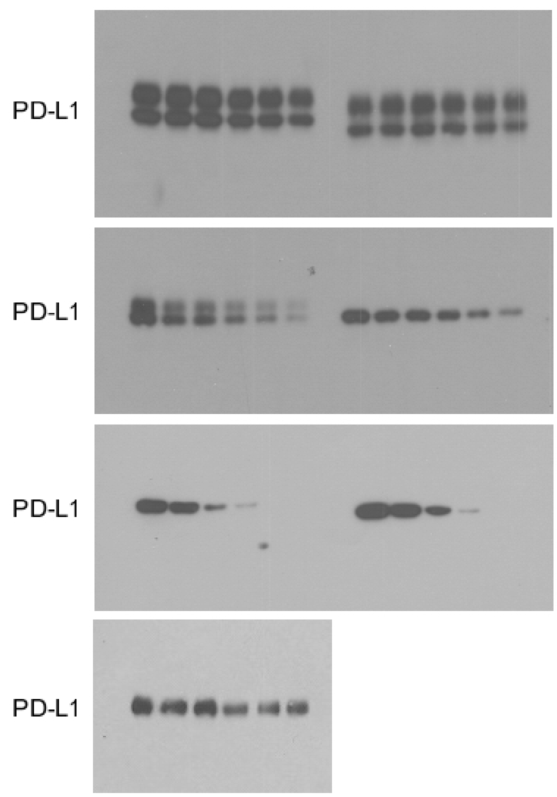

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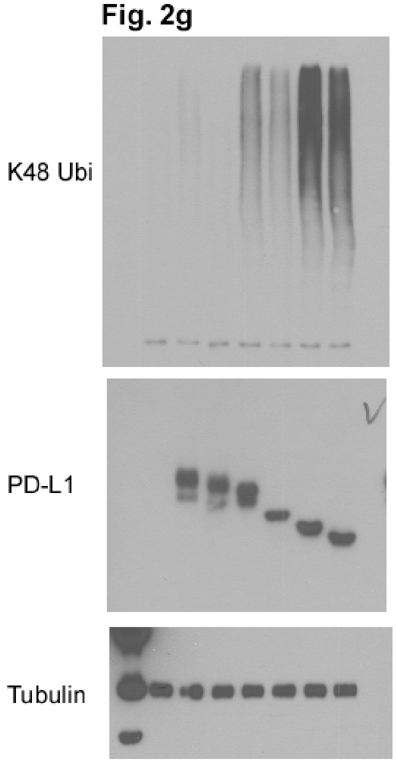

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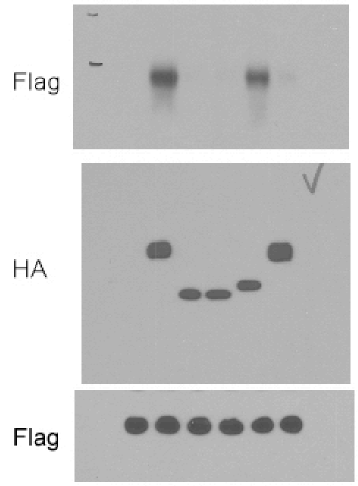


Fig. 3d

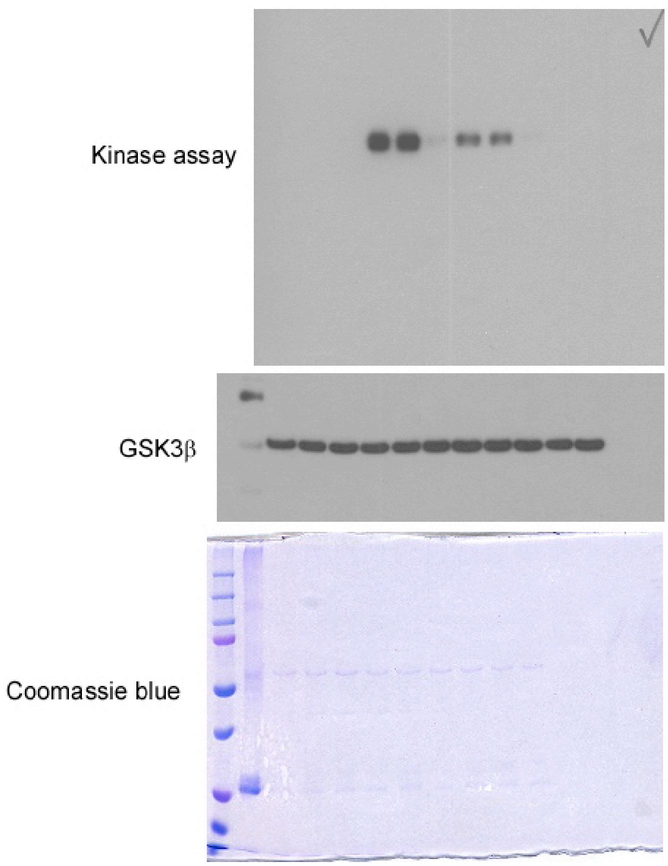


Fig. 3e

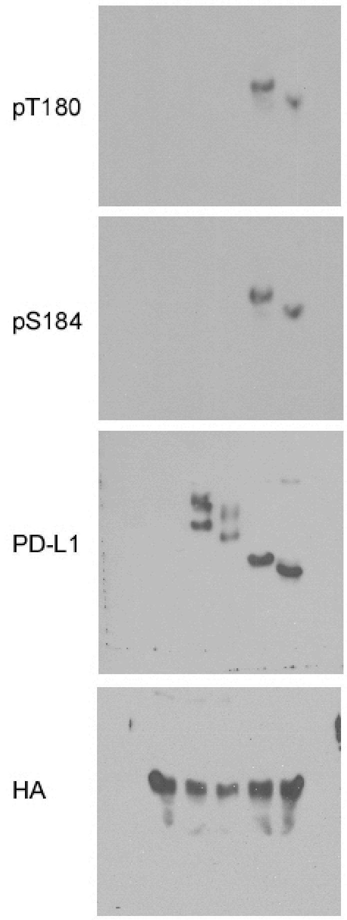
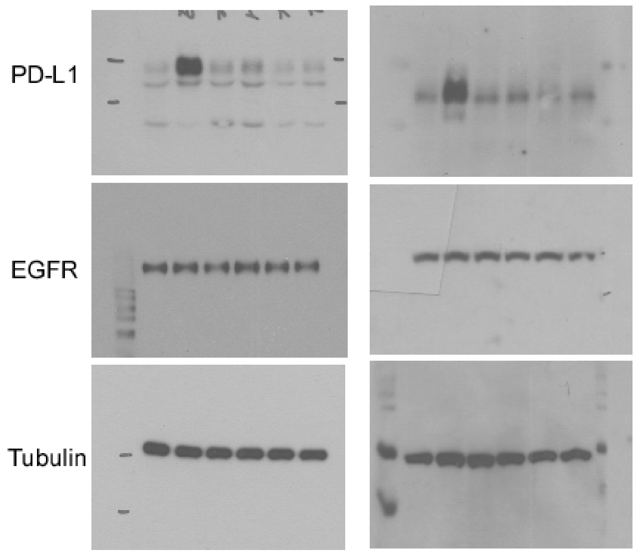


Fig. 4a



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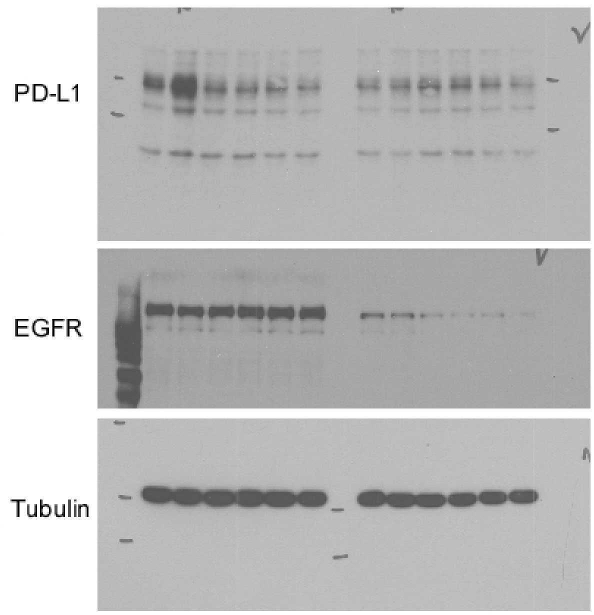


Fig. 4e

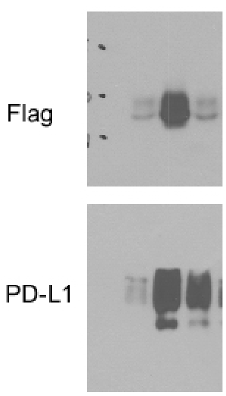


Fig. 4d

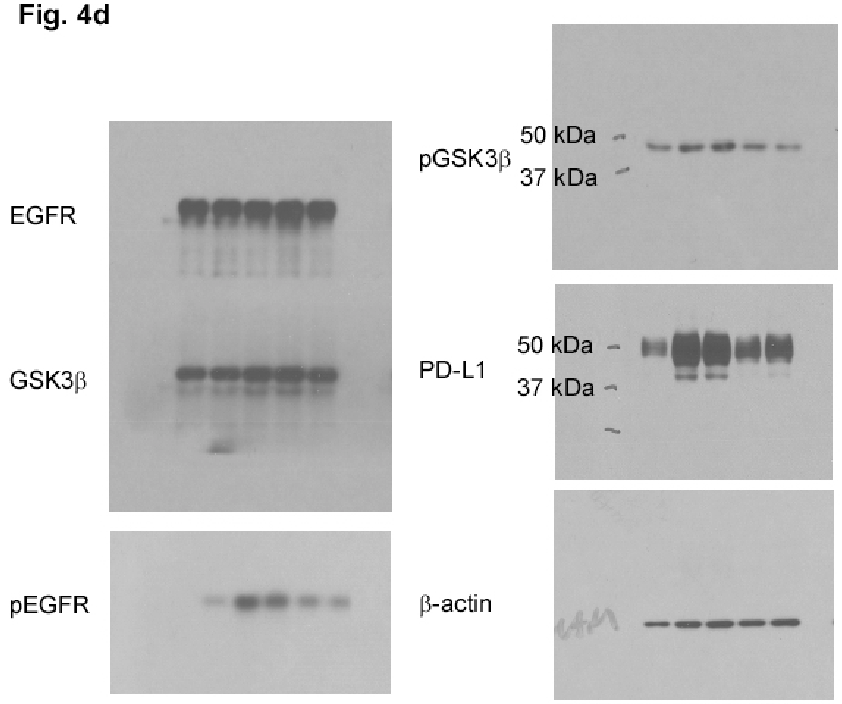


Fig. 5a

pEGFR

PD-L1

β-actin

Fig. S1a

PD-L1

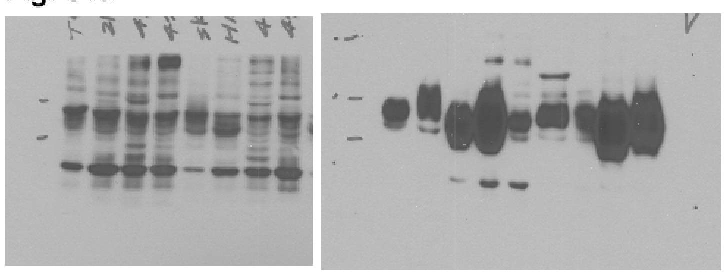
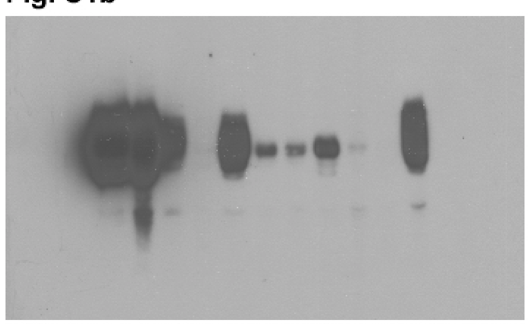


Fig. S1b

PD-L1



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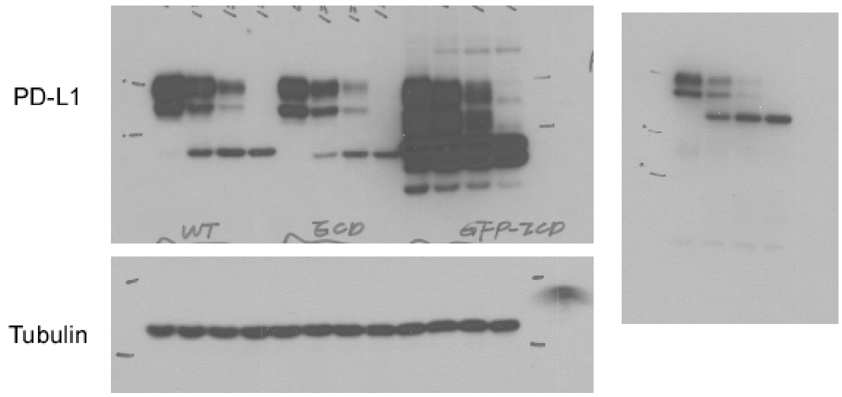

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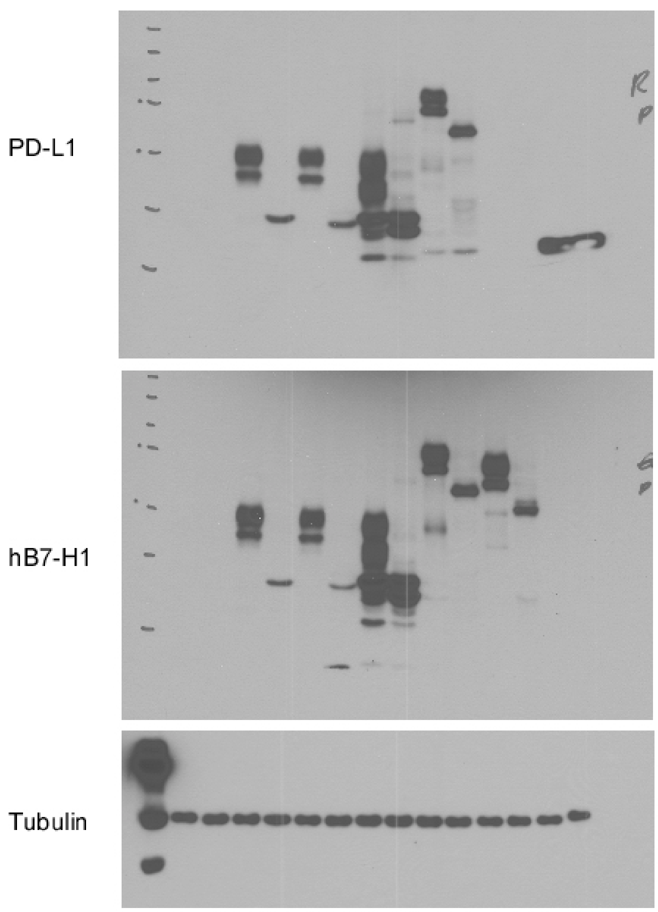

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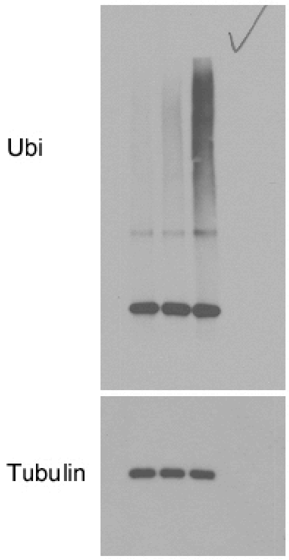

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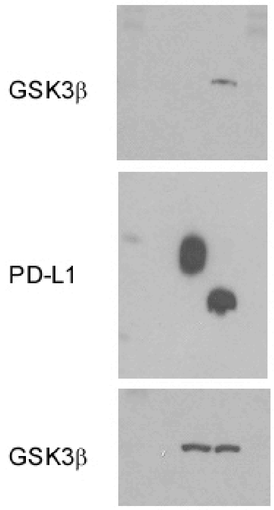

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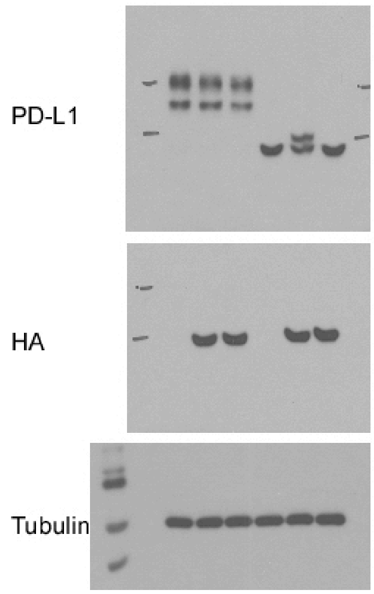


Fig. S5g

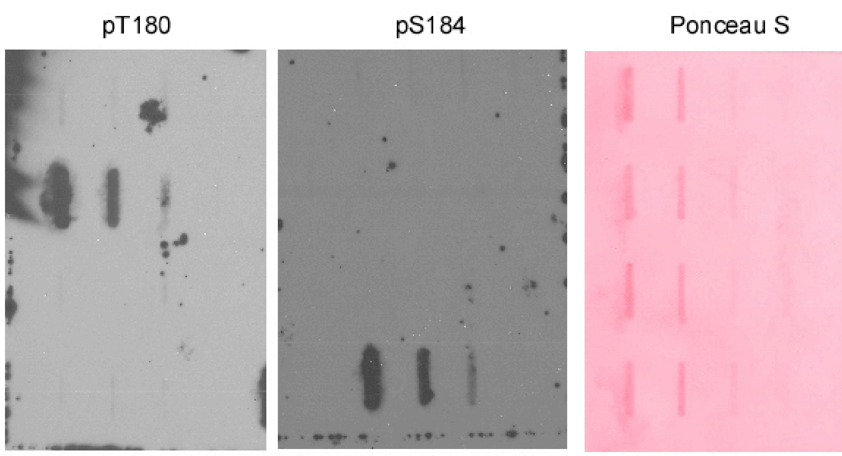


Fig. S6a

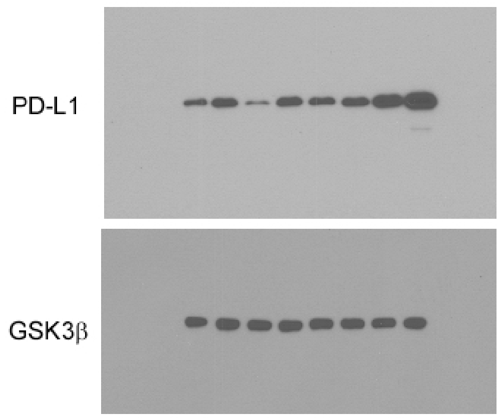
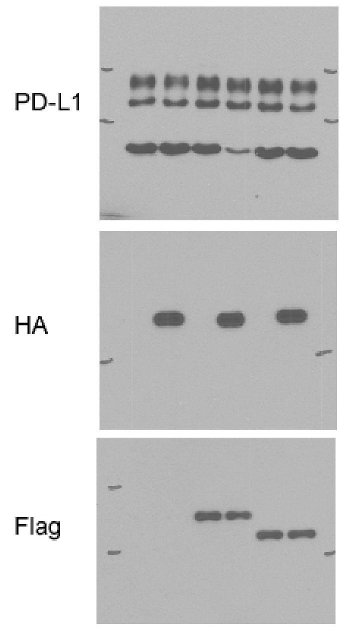


Fig. S6c



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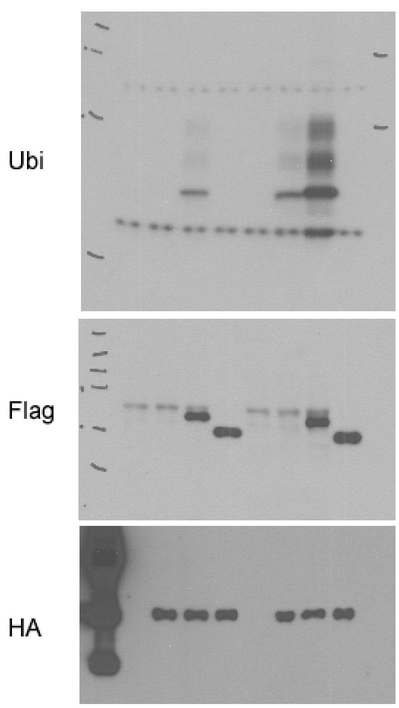

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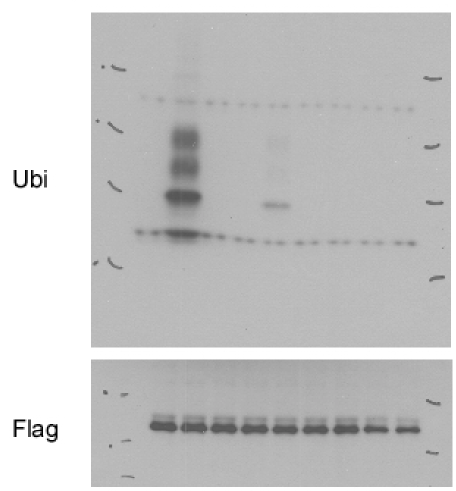

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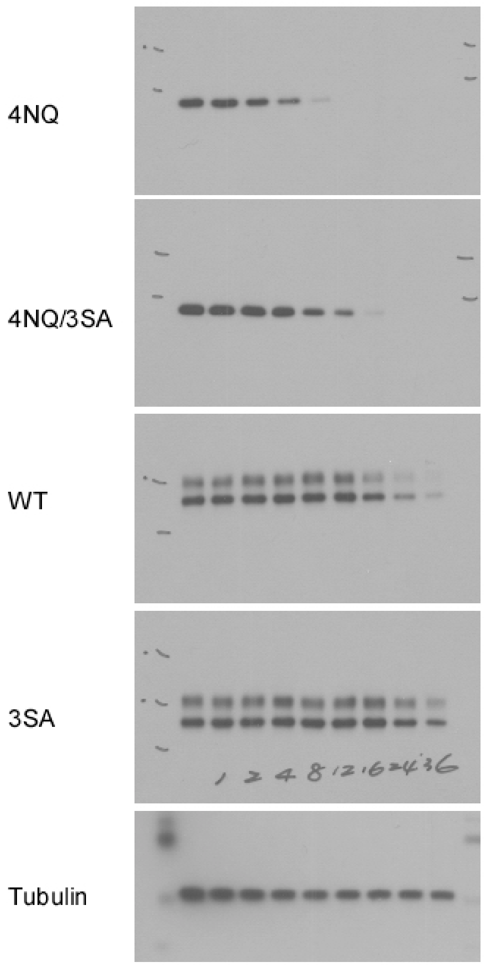
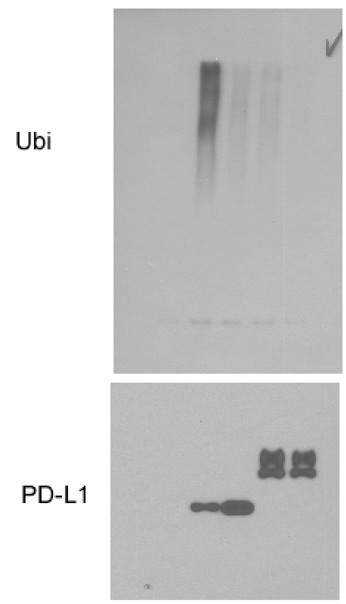


Fig. S7h



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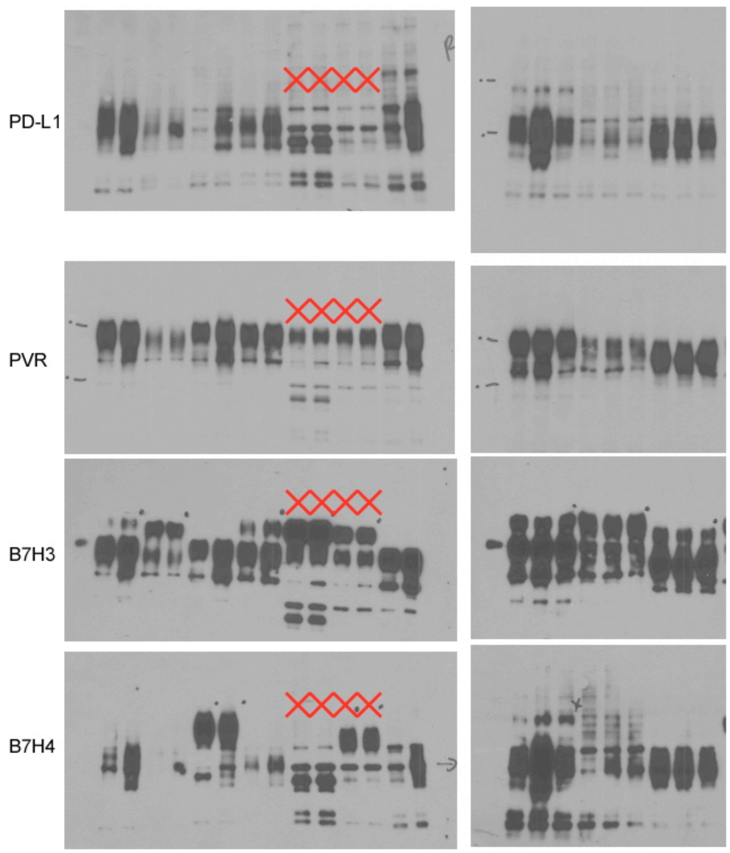
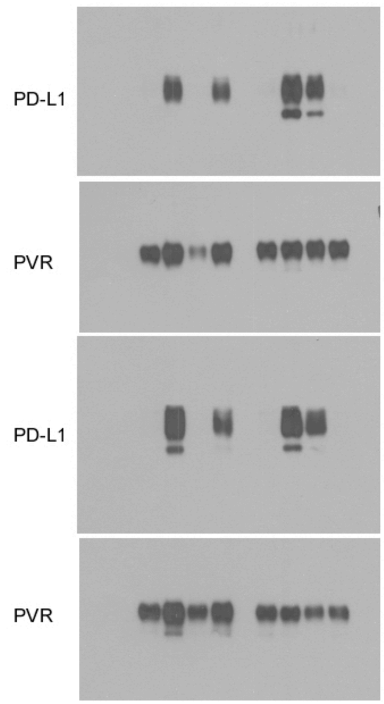


Fig. S8b



Supplementary Figure 10. Uncropped scans of the Western blots shown in the indicated figures.

Supplementary Table 1. Correlations between expression levels of PD-L1, p-EGFR (Tyr 1068), p-GSK3β (Ser 9), and granzyme B expression in surgical specimens of breast cancer.

		No. expression of PD-L1 (%)			
		− / +	++ /+++	Total	*P* value
p-EGFR	−/ +	26 (45.6%)	28 (25.2%)	54 (32.1%)	
	++ /+++	31 (54.4%)	83 (74.8%)	114 (67.9%)	*P* = 0.007
	Total	57 (100%)	111 (100%)	168 (100%)	
p-GSK3β	− / +	27 (42.9%)	4 (5.2%)	31 (22.1%)	
	++ /+++	36 (57.1%)	73 (94.8%)	109 (77.9%)	*P* = 0.0001
	Total	63 (100%)	77 (100%)	140 (100%)	
Granzyme B	−/ +	50 (68.5%)	92 (81.4%)	142 (76.3%)	
	++ /+++	23 (31.5%)	21 (18.6%)	44 (23.7%)	*P* = 0.043*
	Total	73 (100%)	113 (100%)	186 (100%)	

P, Pearson Chi-Square test; *inverse correlation between PD-L1 and granzyme B. –/+, negative or low expression; ++/+++, medium or high expression.