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Somatic PDGFRB activating variants in fusiform cerebral aneurysms

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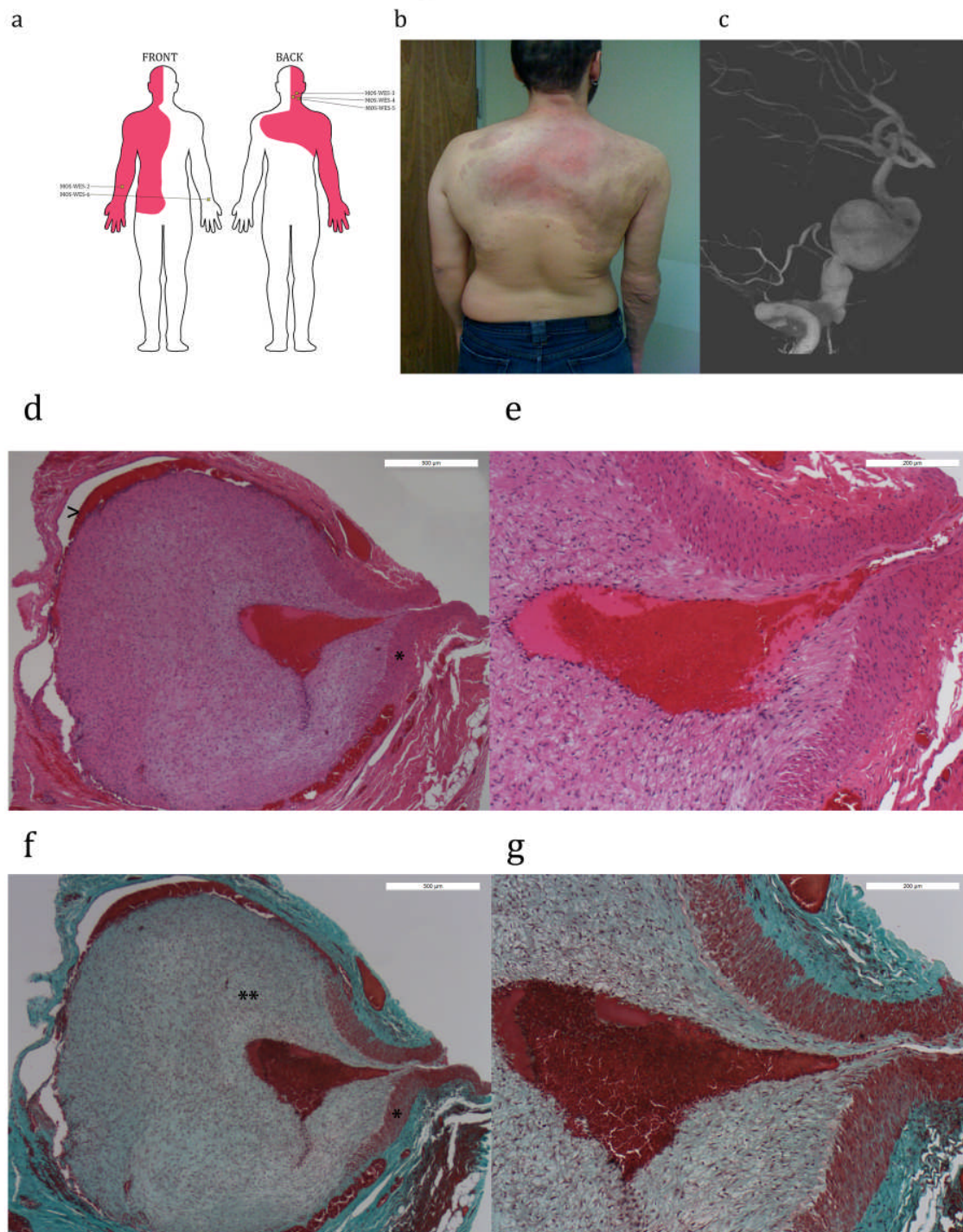
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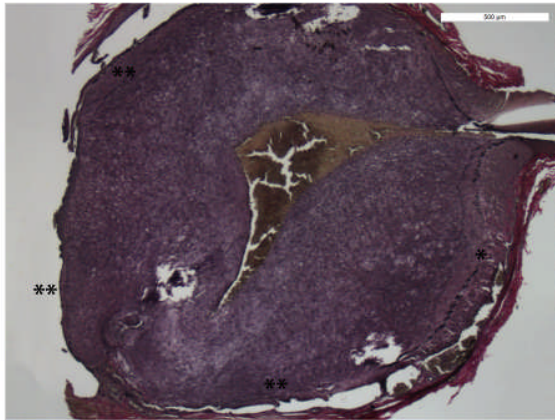
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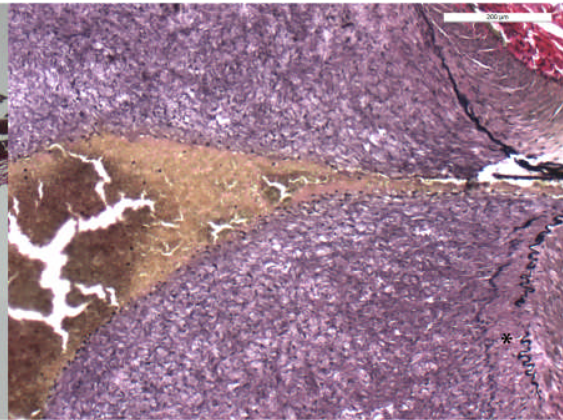
Figure 1



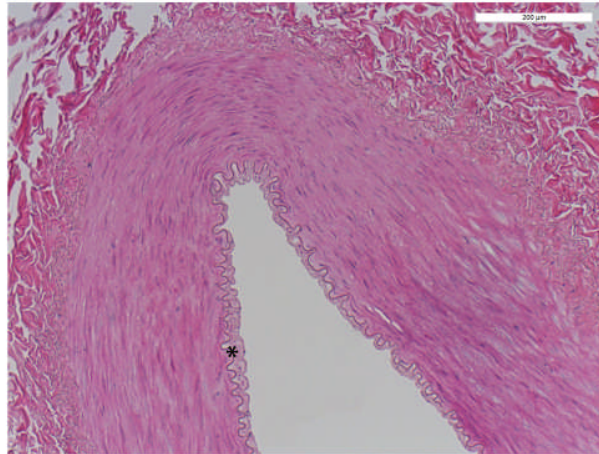
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i



j



k

Sample ID	Anatomic Location	Read Depth (Variant / Total)	Allele Fraction (%)
MOS-WES-1	Peripheral blood	0 / 90	0.00
MOS-WES-2	Cultured fibroblasts from area of mosaicism	32 / 83	38.55
MOS-WES-3	Occipital artery from area of mosaicism	24 / 45	53.33
MOS-WES-4	Soft tissue #1 from area of mosaicism	35 / 114	30.70
MOS-WES-5	Soft tissue #2 from area of mosaicism	6 / 32	18.75
MOS-WES-6	Healthy radial artery from outside area of mosaicism	0 / 43	0.00

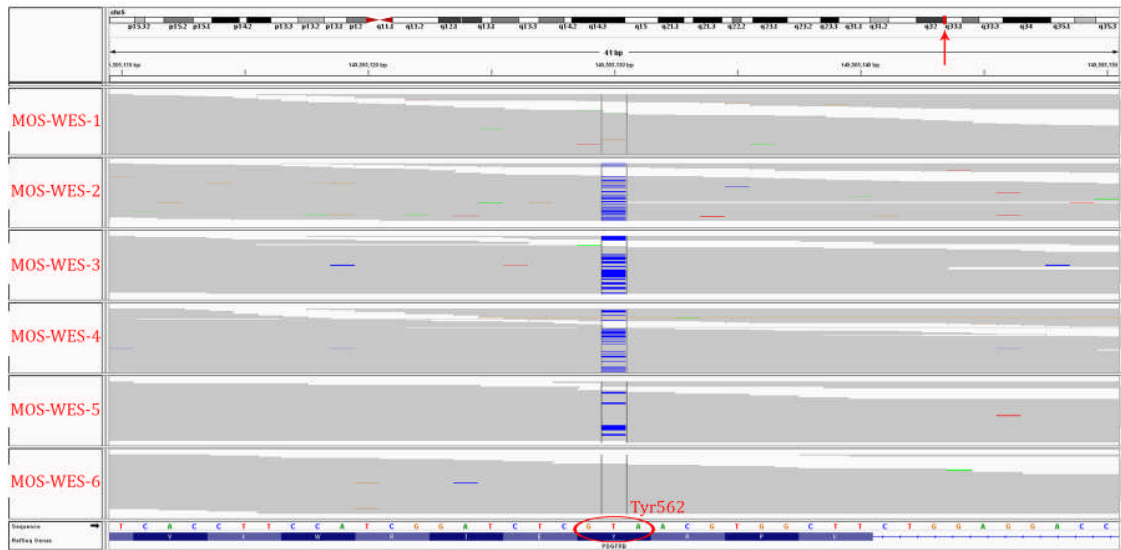


Figure 1: Index patient phenotype and PDGFRB mutation. **a**, Body map showing region of skin mosaicism (in red) and regions of tissue specimens used for exome sequencing. **b**, Cutaneous manifestations and appearance. **c**, Angiogram 3D reconstruction of right vertebral injection illustrating a giant vertebral fusiform aneurysm. **d-i**, Occipital artery aneurysm (Specimen MOS-WES-3): **d**, Low power (4x) and **e**, medium power (10x) magnification of a hematoxylin and eosin (H&E) stained slide demonstrating an aneurysmal dilatation with evidence of dissection (arrowhead). There is severe intimal hyperplasia while the tunica media (*) becomes markedly attenuated. **f**, Low power (4x) and **g**, medium power (10x) magnification of Gomori trichrome (GT) stained slide showing similar features as those noted on the H&E with the intima (**) and the tunica media (*) highlighted. **h**, Low power (4x) and **i**, medium power (10x) magnification of Verhoeff-Van Gieson (VVG) stain stained slide showing similar features as those noted on H&E and GT stains. The internal elastic lamina (*) associated with the relatively better preserved fragment of tunica media, as well several areas with attenuated internal elastic lamina along the aneurysm are highlighted (**). **j**, Specimen MOS-WES-6, medium power magnification (10x) of a H&E stained slide showing an unremarkable left arm radial artery. Well-defined intimal layer, tunica media, and tunica adventitia along with an intact internal elastic lamina (*) are visualized. **k**, Table summarizing the six specimens used for exome sequencing from the index patient and the

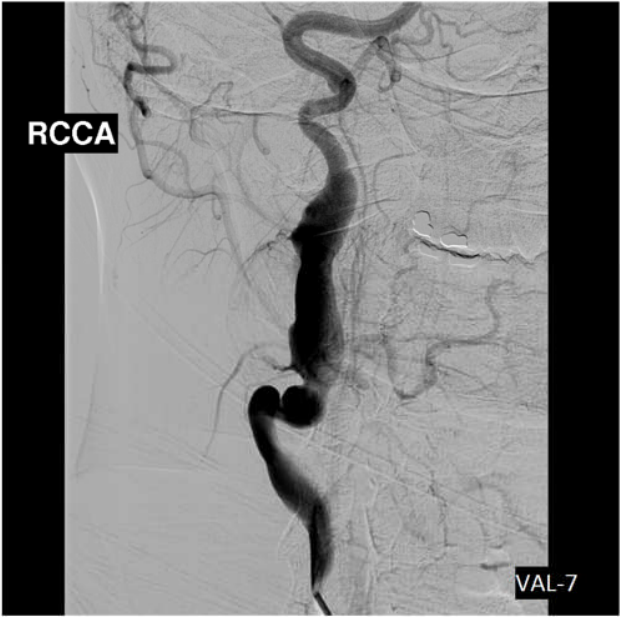
sequencing coverage of the Y562C variant. **1**, Next generation sequencing reads across the area of the missense mutation, with the variant nucleotides (C) in blue. The reference nucleotide and amino acid sequences are at the bottom.

Figure 2

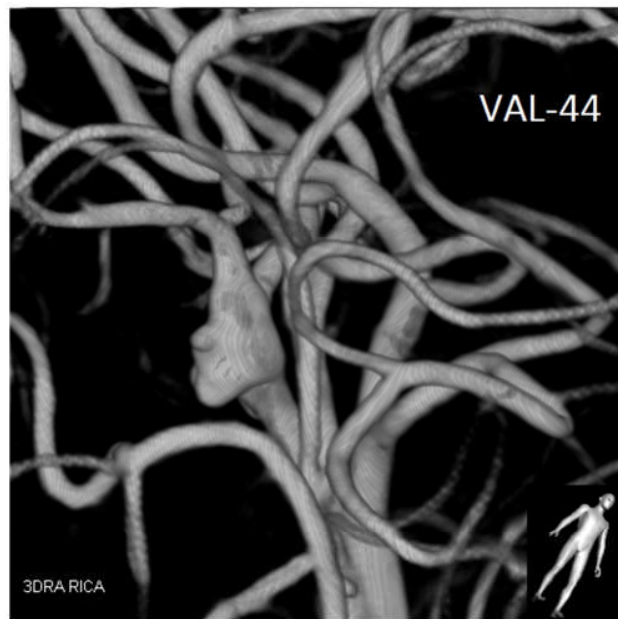
a

ID	Age	Gender	Ethnicity	Aneurysm	Genomic Position	cDNA	Protein	AF
<u>Index</u>	<u>23</u>	<u>Male</u>	<u>Caucasian</u>	<u>Multiple fusiform</u>	<u>chr5:g.149505130T>C</u>	<u>c.1685A>G</u>	<u>p.Tyr562Cys</u>	<u>18.75%-53.33%</u>
Val-7	40	Male	Caucasian	3cm R cervical ICA fusiform	chr5:149500456 CT > C & chr5:149500483 TGATGTCTC > T	c.2546_2553delGAGACATC & c.2580delA	p.Arg849_Lys860delinsHAGLEHLQ	9.82% & 8.59%
VAL-44	7	Female	Chinese	8mm R MCA fusiform	chr5:149500489 C > A	c.2548G>T	p.Asp850Tyr	5.57%
VAL-62	17	Female	Unknown	3cm R PCA fusiform	chr5:149505118 CATCGGATCTCGT > C	c.1685_1696delACGAGATCCGAT	p.Tyr562_Arg565del	15.17%

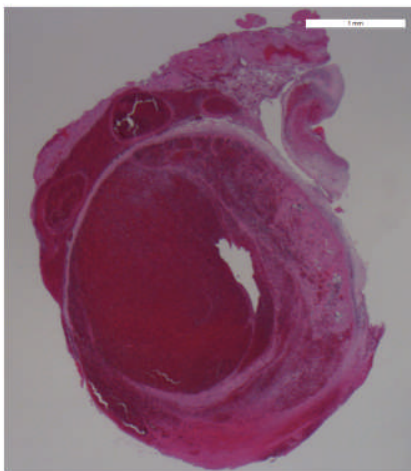
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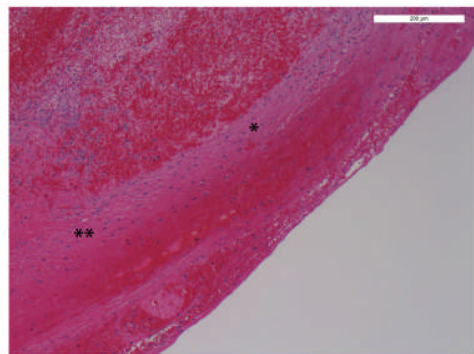
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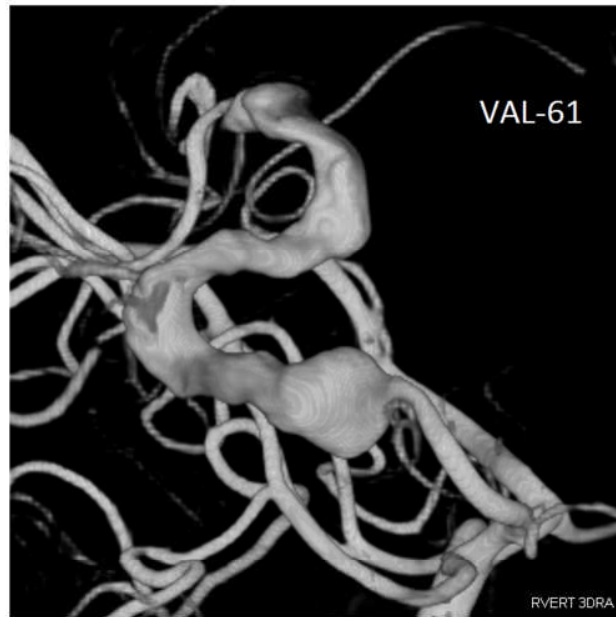
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e



f



g

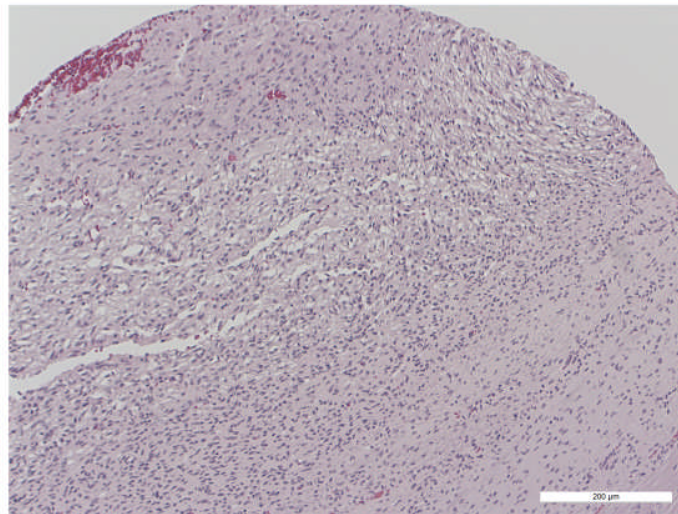
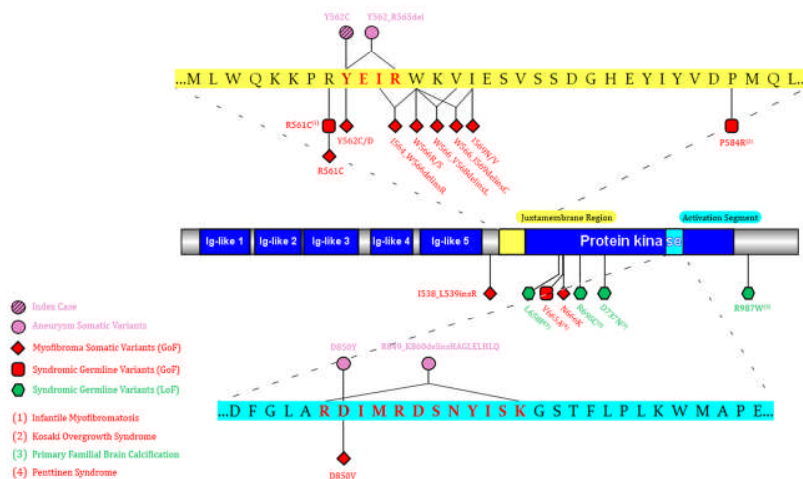


Figure 2: Sporadic fusiform aneurysms harbor PDGFRB mutations. **a**, Demographics of sporadic fusiform aneurysm subjects and variants. **b, c, f**, Angiogram and/or 3D representative images illustrating the fusiform morphology. **d** and **e**, Representative H&E stained sections of specimen VAL-44 at low (1.25x) and medium (10x) magnification showing a markedly affected vessel with vascular wall attenuation, an intraluminal thrombus with early organization and dissecting hemorrhage. The tunica media (*) is focally present and becomes attenuated (**). **g**, Medium magnification (10X)

tissue micro array (TMA) punch of specimen VAL-61 stained with H&E showing a small portion of arterial wall with marked intimal proliferation displacing the internal elastic lamina (not shown). Abbreviations: AF = Allele frequency, ICA = Internal carotid artery, MCA = Middle cerebral artery, PCA = Posterior cerebral artery, RCCA = Right common carotid artery.

Figure 3

a



b

521	531	541	551	561	571	581	KIT
FTPLLI GFVI	VAGMMCIIVM	ILTYKYLQKP	<u>MYE</u> VOQKWVE	EINGNNYVYI	DPTQLPYDHK	WEFPRNRLSF	
524	534	544	554	564	574	584	PDGFRA
ELTVAAAVLV	LLVIVIISLI	VLVVIWKQKP	<u>RYE</u> IRWRVIE	SISPDGHEYI	YVDPMQLPYD	SRWEFPRDGL	
531	541	551	561	571	581	591	PDGFRB
KVVVISAILA	LVLVTIISLI	ILIMLWQKKP	<u>RYE</u> IRWKVIE	SVSSDGHEYI	YVDPMQLPYD	STWELPRDQL	
531	541	551	561	571	581	591	PDGFRB - Index Case
KVVVISAILA	LVLVTIISLI	ILIMLWQKKP	<u>RCE</u> IRWKVIE	SVSSDGHEYI	YVDPMQLPYD	STWELPRDQL	
531	541	551	561	571	581	591	PDGFRB - VAL-61
KVVVISAILA	LVLVTIISLI	ILIMLWQKKP	R- <u>---</u> WKVIE	SVSSDGHEYI	YVDPMQLPYD	STWELPRDQL	

C

810	DFGLA <u>RD</u> IKNDSNYVVKGNARLPVKWMAPE	839	KIT
836	DFGLA <u>RD</u> IMHDSNYVSKGSTFLPVKWMAPE	865	PDGFRA
844	DFGLA <u>RD</u> IMRDSNYISKGSTFLPLKWMAPE	873	PDGFRB
844	DFGLA <u>RY</u> IMRDSNYISKGSTFLPLKWMAPE	873	PDGFRB - VAL-44
844	DFGLA <u>HAGLELHLQ</u> GSTFLPLKWMAPE	873	PDGFRB - VAL-7

d

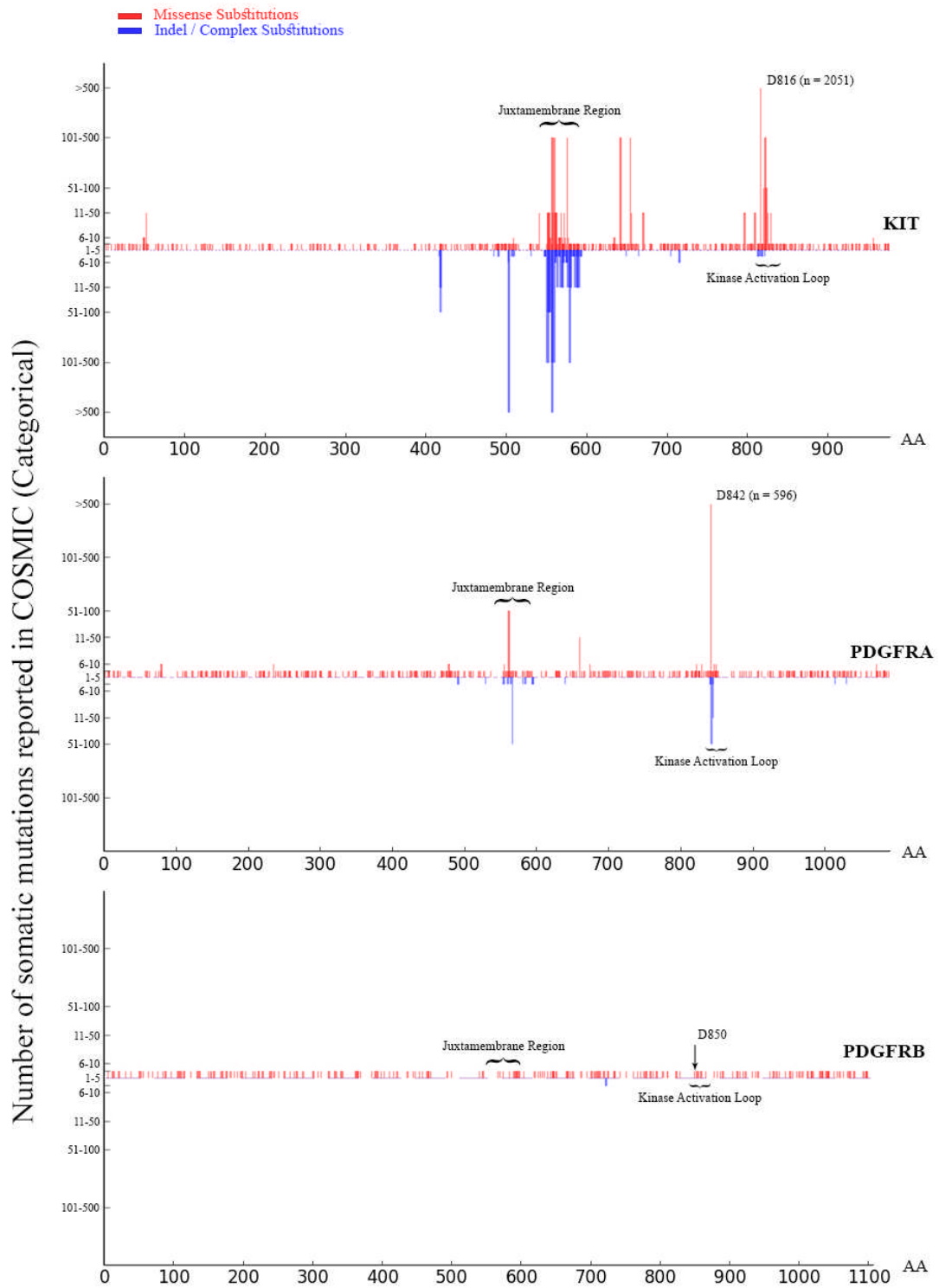
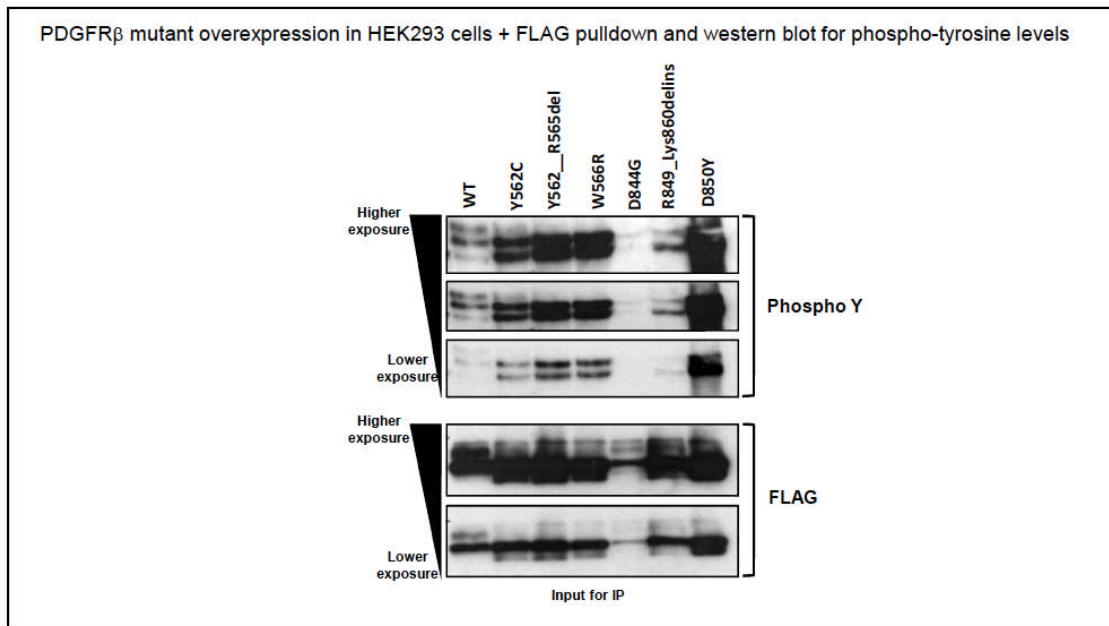


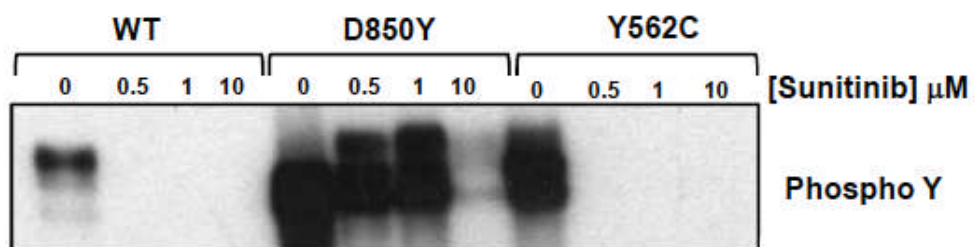
Figure 3: Mutations in PDGFRB within the juxtamembrane region and the kinase activation loop found in fusiform aneurysms. **a**, Schematic representation of the PDGFRB protein, amino acid sequence of the two hotspots, and the location of variants. Germline and somatic PDGFRB variants with known or implied functional consequences in other syndromes and diseases included for comparison. **b**, Homologous juxtamembrane amino acid sequences for KIT, PDGFRA and PDGFRB and location of aneurysm mutations. **c**, Homologous kinase domain activation loop amino acid sequences for KIT, PDGFRA and PDGFRB and location of aneurysm mutations. **d**, All somatic variants reported in the COSMIC database with possible activating consequences (missense and in-frame insertion/deletions) in KIT, PDGFRA and PDGFRB. Notice the increased frequency of variants in both juxtamembrane region and kinase activation loop of KIT and PDGFRA. There is a comparative lack of variants reported in PDGFRB.

Figure 4

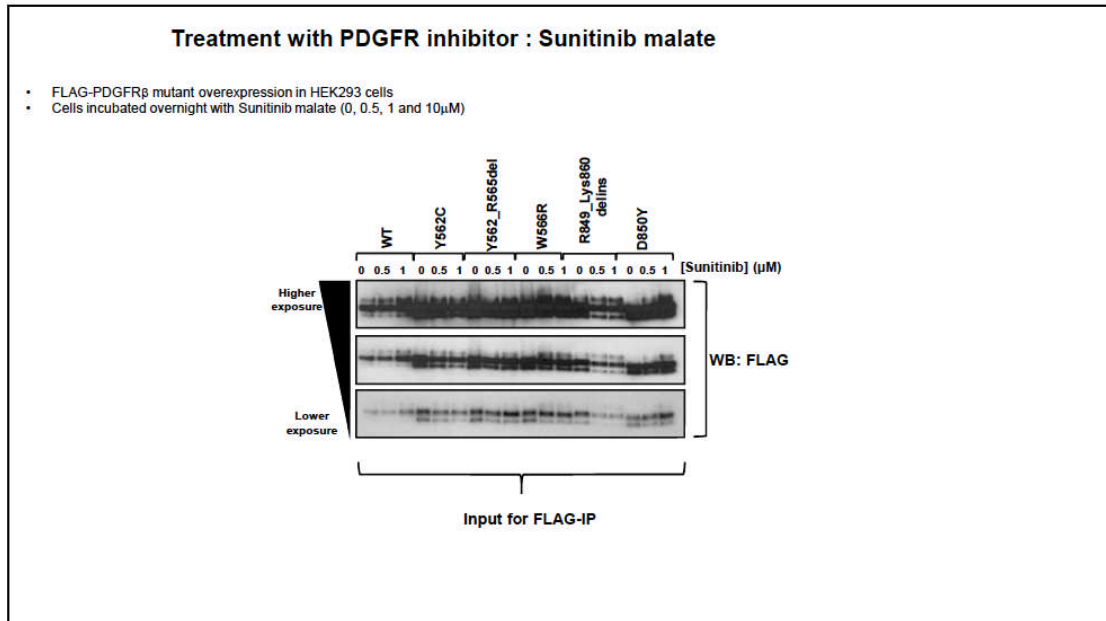
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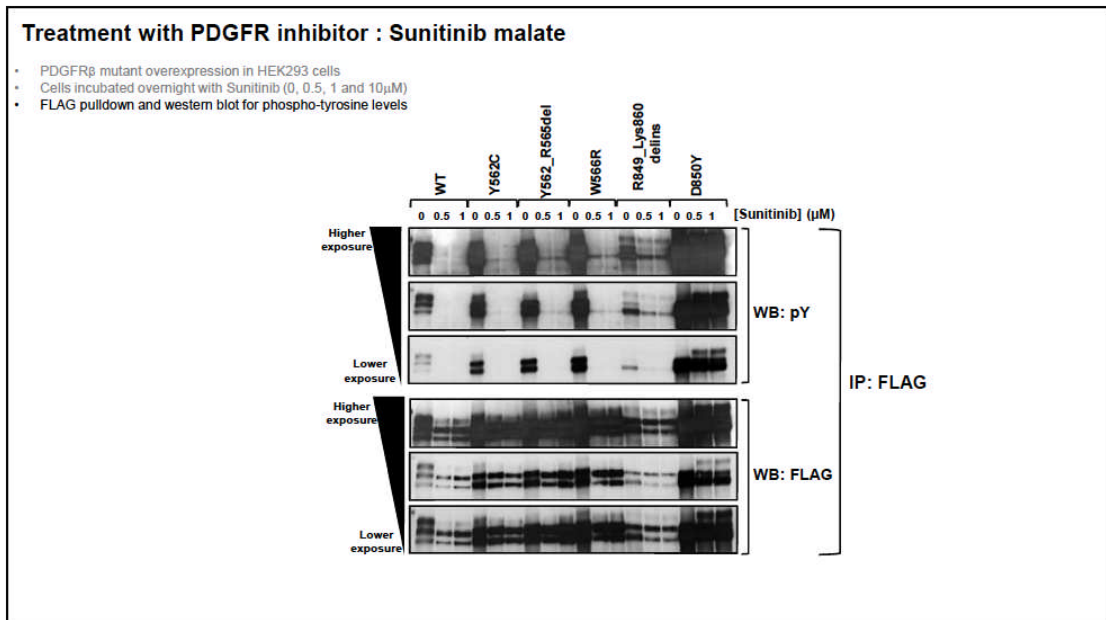
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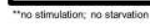
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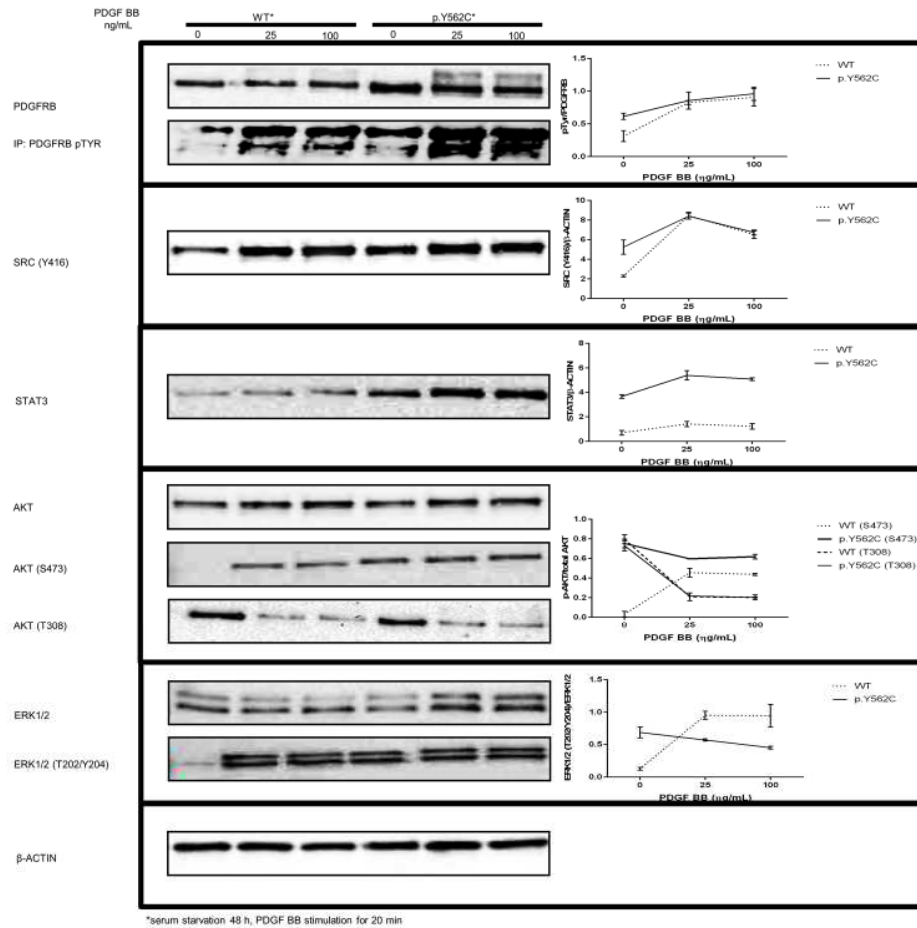


Figure 4: PDGFRB variants are constitutively phosphorylated, sensitive to sumatinib kinase inhibition and activate downstream signaling pathways. a, Phosphorylation and expression of wild-type and mutated receptors by western blot. HEK cells stably expressing aneurysm variants, W566R (IM - gain of function) and D844G (Fahr's Disease - loss of function) variants. **b,** Sensitivity of WT, D850Y and Y562C aneurysm variant PDGFRB auto-phosphorylation to sumatinib. **c** and **d,** Aneurysm PDGFRB and auto-phosphorylation sensitivity to sumatinib. **e,** western blot analysis of normal and mosaic affected non-starved fibroblast cells expressing wild-type and Y562C

PDGFRB variants from index patient. **f**, Western blot analysis of PDGFbb stimulated normal and mosaic starved fibroblast cells expressing wild-type and Y562C PDGFRB variants from index patient.