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Supplementary Materials for

The role of H3K36 methylation and associated methyltransferases in chromosome-specific gene regulation

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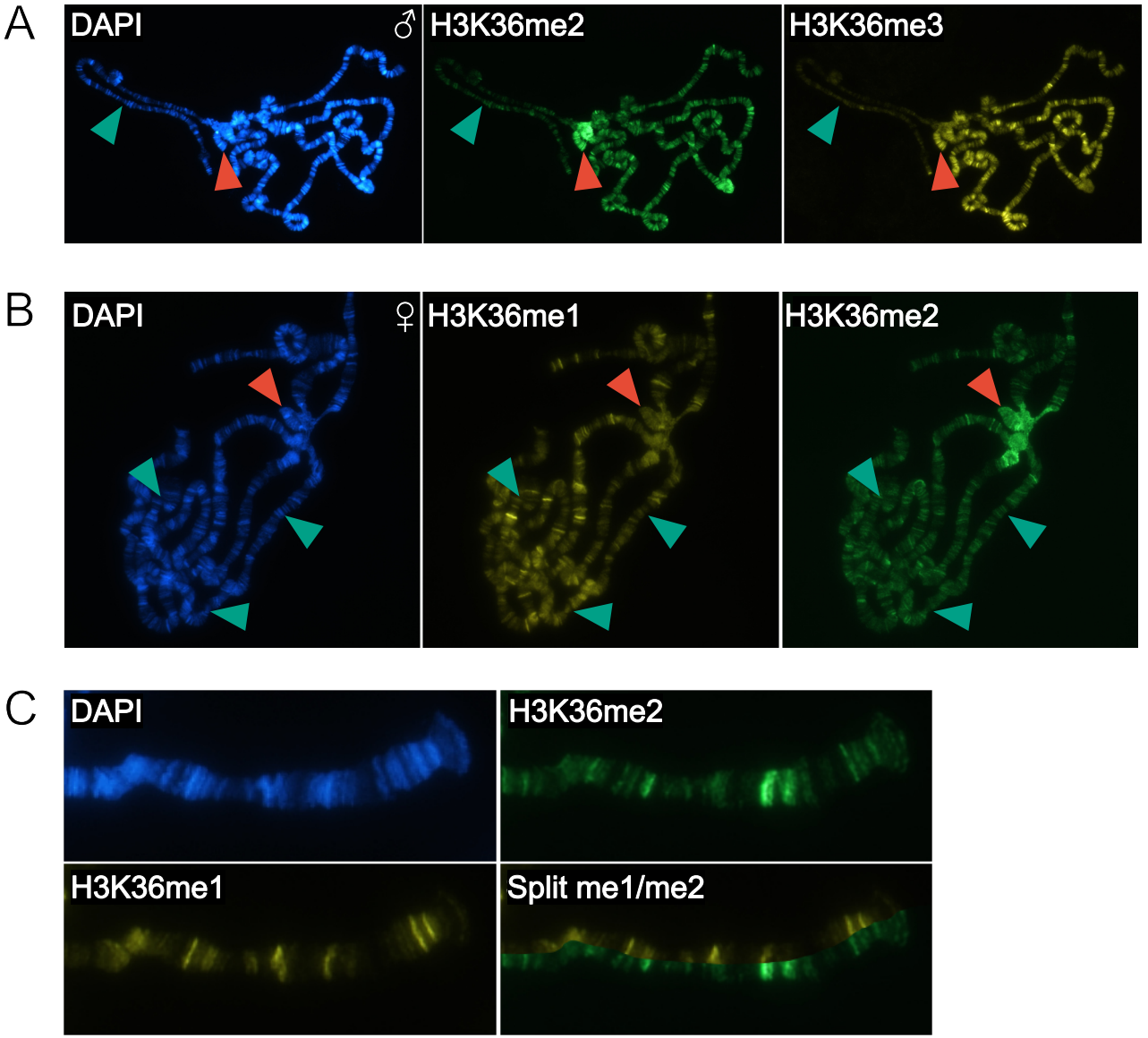

Figure S1. Differential enrichments of methylated H3K36. (**A**) Immunostaining of male third instar larvae polytene chromosomes showing H3K36me2 (green), H3K36me3 (yellow) and DAPI staining of DNA in blue. Note the accumulation of H3K36me2 in pericentromeric heterochromatin, H3K36me3 on the 4th chromosome and the reduced amount of H3K36me3 on the X-chromosome. (**B**) Immunostaining of female third instar larvae polytene chromosome showing H3K36me1 (yellow), H3K36me2 (green) and DAPI staining of DNA in blue. Note the accumulation of H3K36me2 in pericentromeric heterochromatin. Chromosome 4 is marked with red arrows and chromosome X with green arrows. (**C**) The H3K36me2 (green) staining shows only minor overlaps with H3K36me1 (yellow).

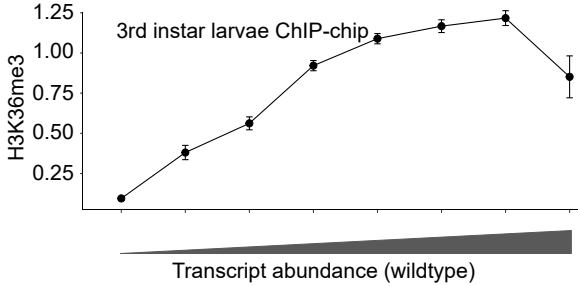

Figure S2. Gene levels of H3K36me3 ChIP signal positively correlate with expression levels. Third instar larvae ChIP-chip gene exon enrichment level (ChIP over input, log2) plotted against transcript abundance in wildtype male third instar larval brains as determined by the RNA-Seq experiment. Highly transcribed genes show a higher accumulation of H3K36me3 than do genes with lower transcript abundance.

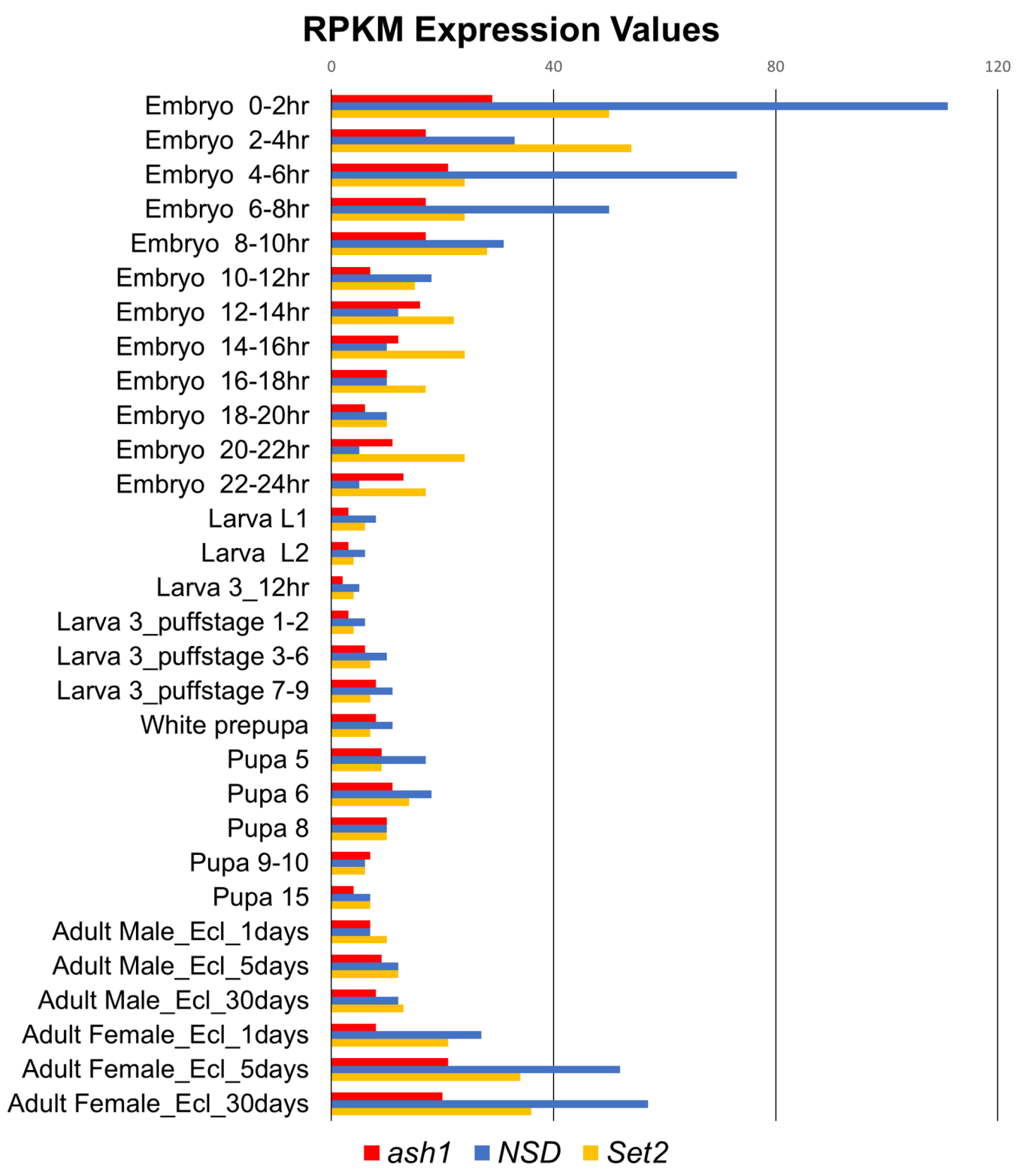

Figure S3. *Set2*, *NSD* and *ash1* show similar developmental RNA profiles. Developmental RNA profiles for *Set2*, *NSD* and *ash1* with the highest levels of RNA maternally contributed and in early embryo stages.

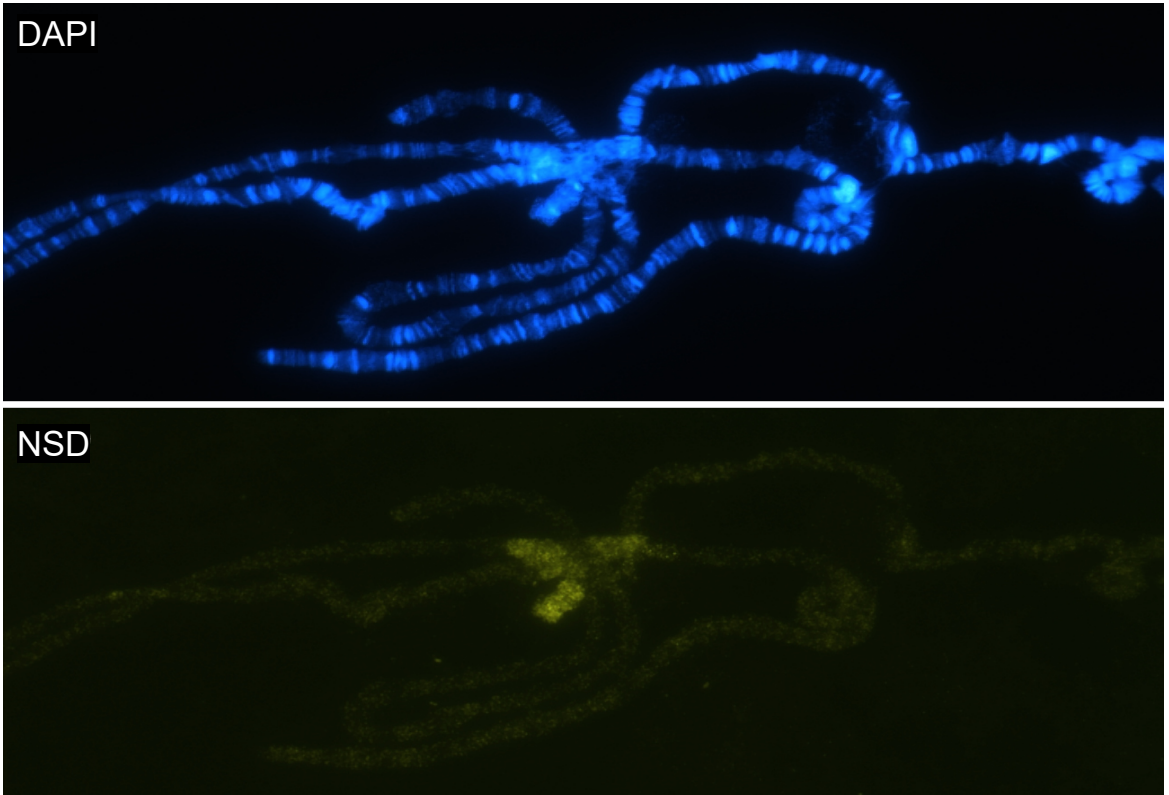

Figure S4. NSD targets pericentric heterochromatin. Immunostaining of third instar larvae polytene chromosome showing NSD (yellow), and DAPI staining of DNA in blue. Note the accumulation of NSD in pericentromeric heterochromatin and region 2L:31, similar to typical enrichments of HP1a.

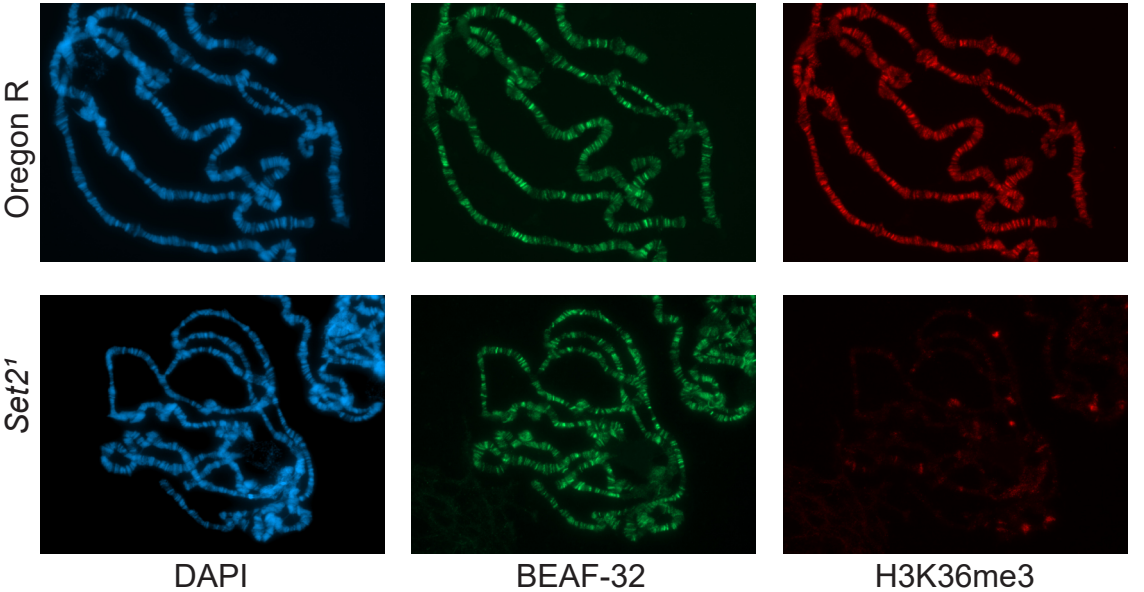

Figure S5. H3K36me3 is lost in *Set2¹* mutants. Staining of H3K36me3 in salivary glands for wildtype (top row) and *Set2¹* (bottom row). Staining of BEAF-32 serves as an internal control.

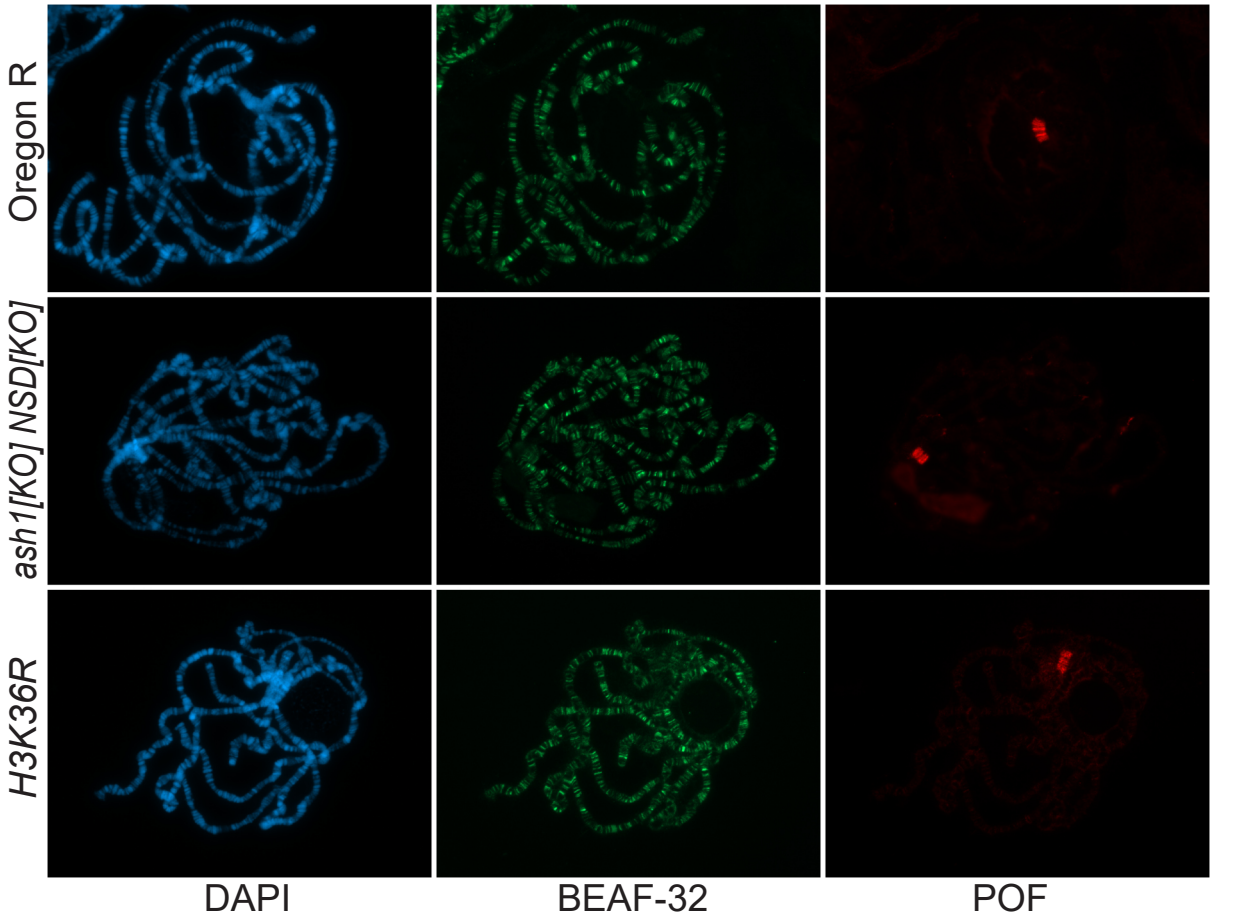

Figure S6. POF binding to the 4th chromosome is unaltered in *ash1 NSD* double mutants and in the *H3K36R* mutant. Staining of POF in salivary glands for wildtype (top row), *ash1*^{22} *NSD*^{ds46}/*ash1*^{9011} *NSD* ^{ds46} (middle row) and *ΔHisC; 12x*^{H3K36R} (bottom row). Staining of BEAF-32 serves as an internal control.

Supplementary table S1: List of antibodies

Antigen	Host	Reference	Polytene chromosomes	Western
H3K36me3	Rabbit monoclonal	Cell Signaling, D5A7, Cat#4909	1:200	1:2000
H3K36me3	Rabbit polyclonal	Abcam, ab9050	1:200	-
H3K36me2	Mouse monoclonal	Active Motifs, MABI 0332	1:50	-
H3K36me1	Rabbit polyclonal	Abcam, ab9048	1:100	-
NSD	Mouse monoclonal	(*39*)	1:10	
POF	Rabbit polyclonal	(*19*)	1:400	
BEAF-32	Mouse monoclonal	DHSB, anti-BEAF	1:5	-
H3	Rabbit polyclonal	Abcam, ab1791	-	1:100 000
Secondary Antibody to Rabbit IgG (H+L), Alexa Fluor® 555 conjugate	Goat polyclonal	Abcam, ab150078	1:500	-
Secondary Antibody to Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor® 488 conjugate	Donkey polyclonal	Invitrogen, A-21202	1:500	-
Secondary Antibody to Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor® 555 conjugate	Donkey polyclonal	Invitrogen, A-31572	1:500	-
Secondary Antibody to Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor® 555	Goat polyclonal	Invitrogen, A-21424	1:500	-
Anti-Rabbit AP conjugated	Goat polyclonal	Promega, S3731	-	1:10000
Anti-Mouse IgG AP conjugated	Goat polyclonal	Sigma, A3562	-	1:10000