

Title: Evaluation of inflammation and follicle depletion during ovarian ageing in mice

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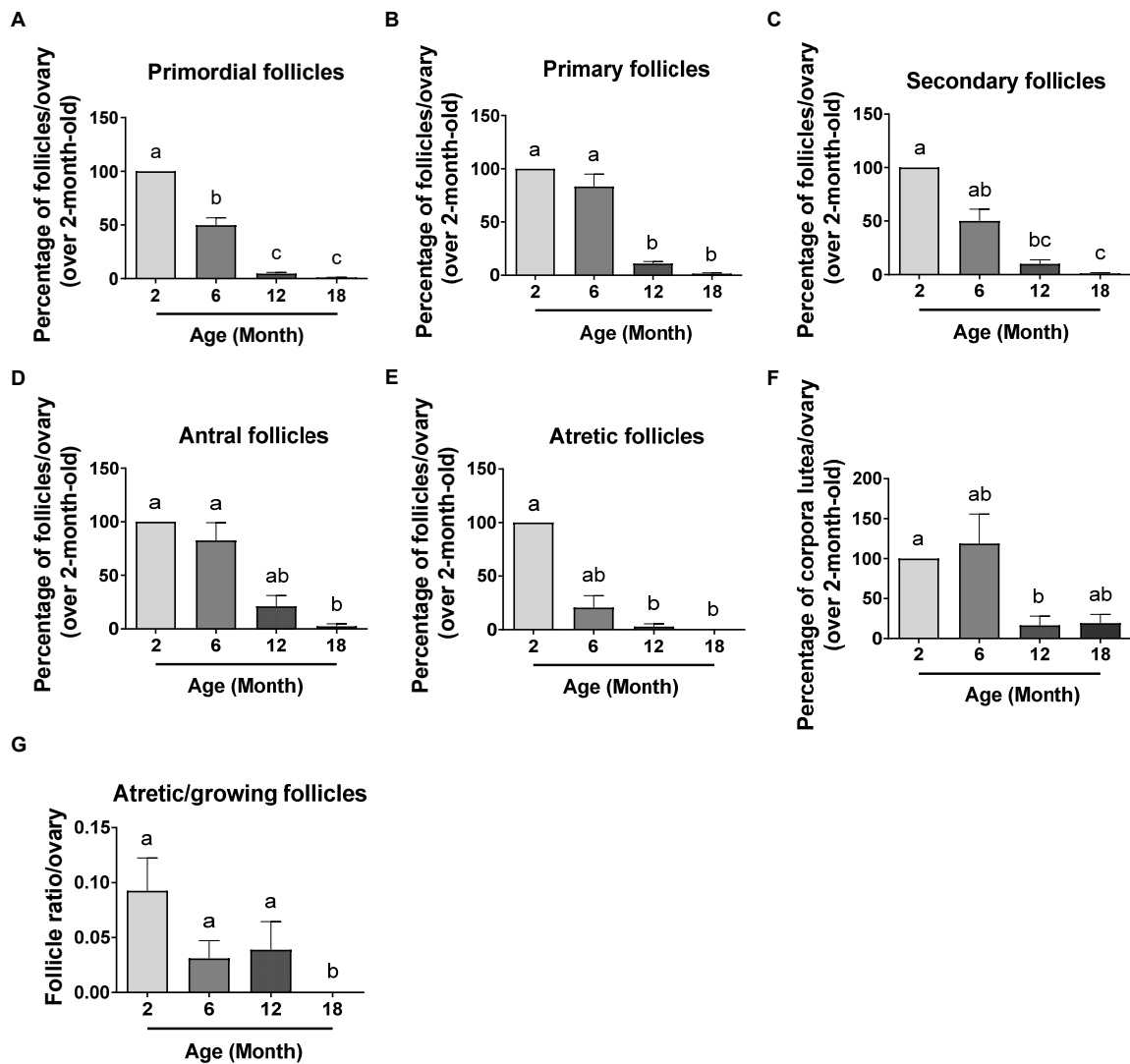
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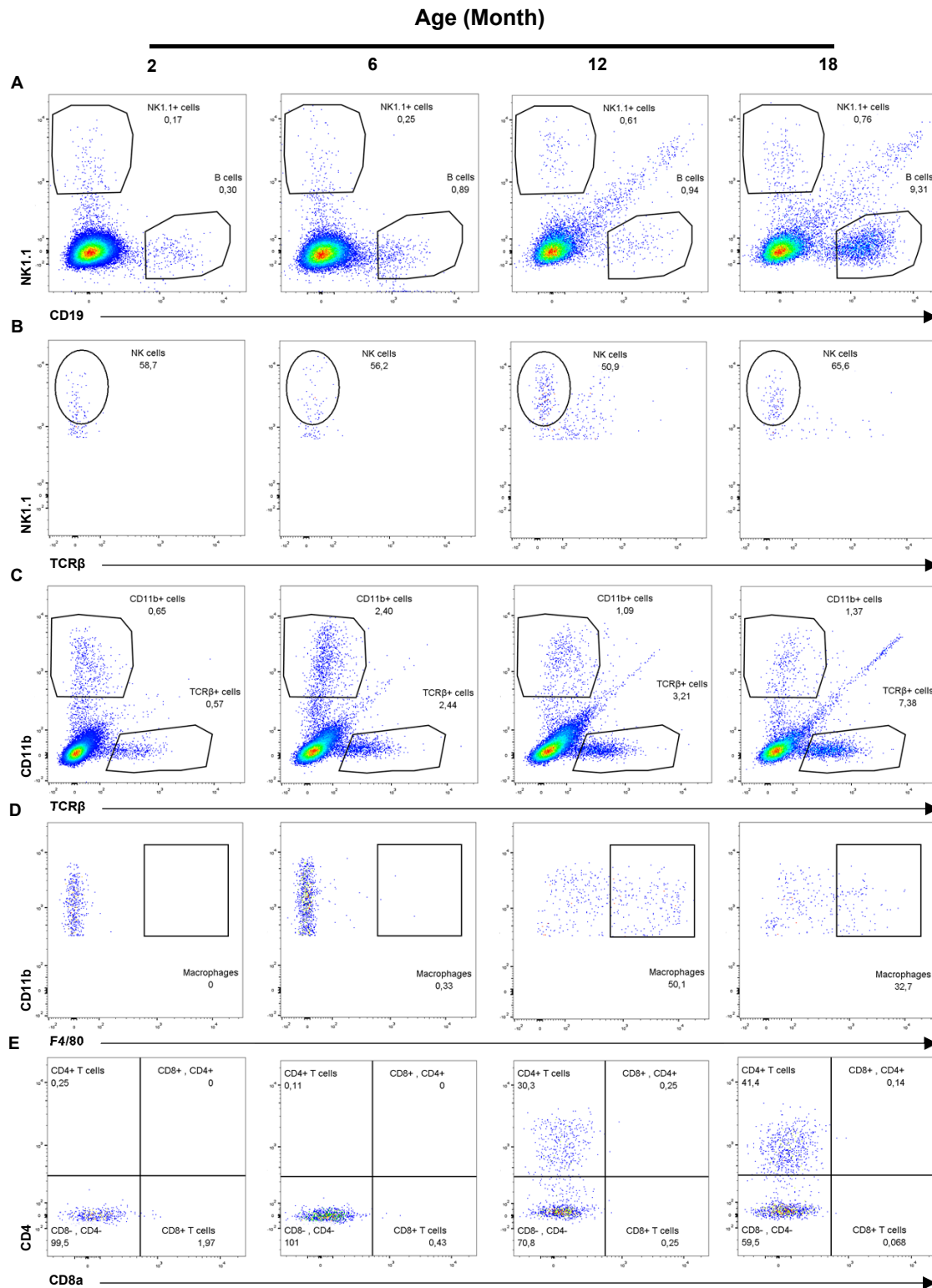
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Supplemental Figure 1



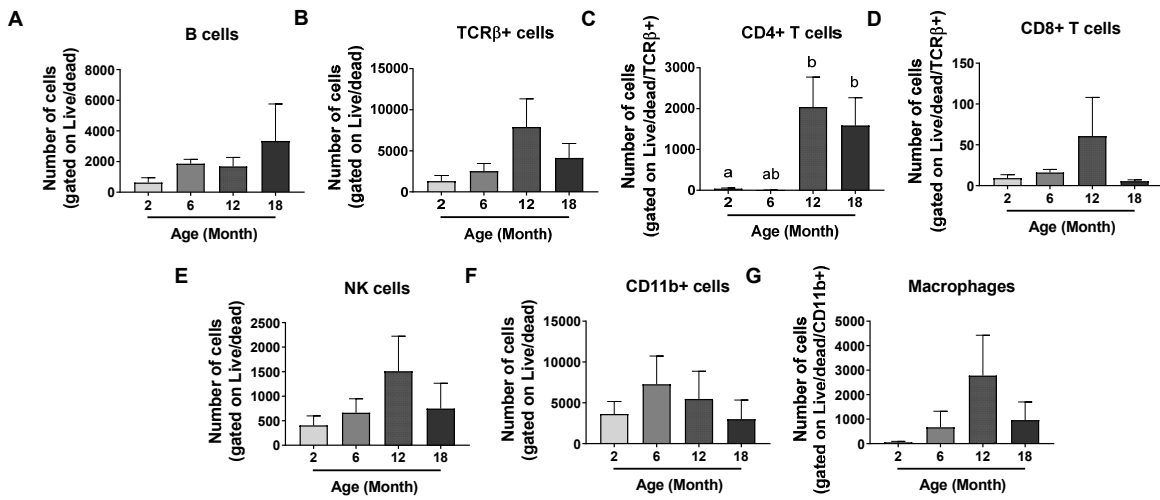
Supplemental Figure 1. Primordial (A), primary (B), secondary (C), antral (D), atretic follicle (E) and corpora lutea (F) percentage (relative to 2-month-old group) and ratio of atretic follicles per total number of growing follicles (secondary and antral follicles) (G) in ovaries from 2, 6, 12 and 18-month-old mice. $n = 6$ per cohort. Data are presented as mean \pm SEM; Kruskal-Wallis test, Dunn's multiple comparisons test: a, b and c are significantly different among groups ($p < 0.05$); Ordinary One-way ANOVA, Tukey's multiple comparisons test: a, b and c are significantly different among groups ($p < 0.05$).

Supplemental Figure 2



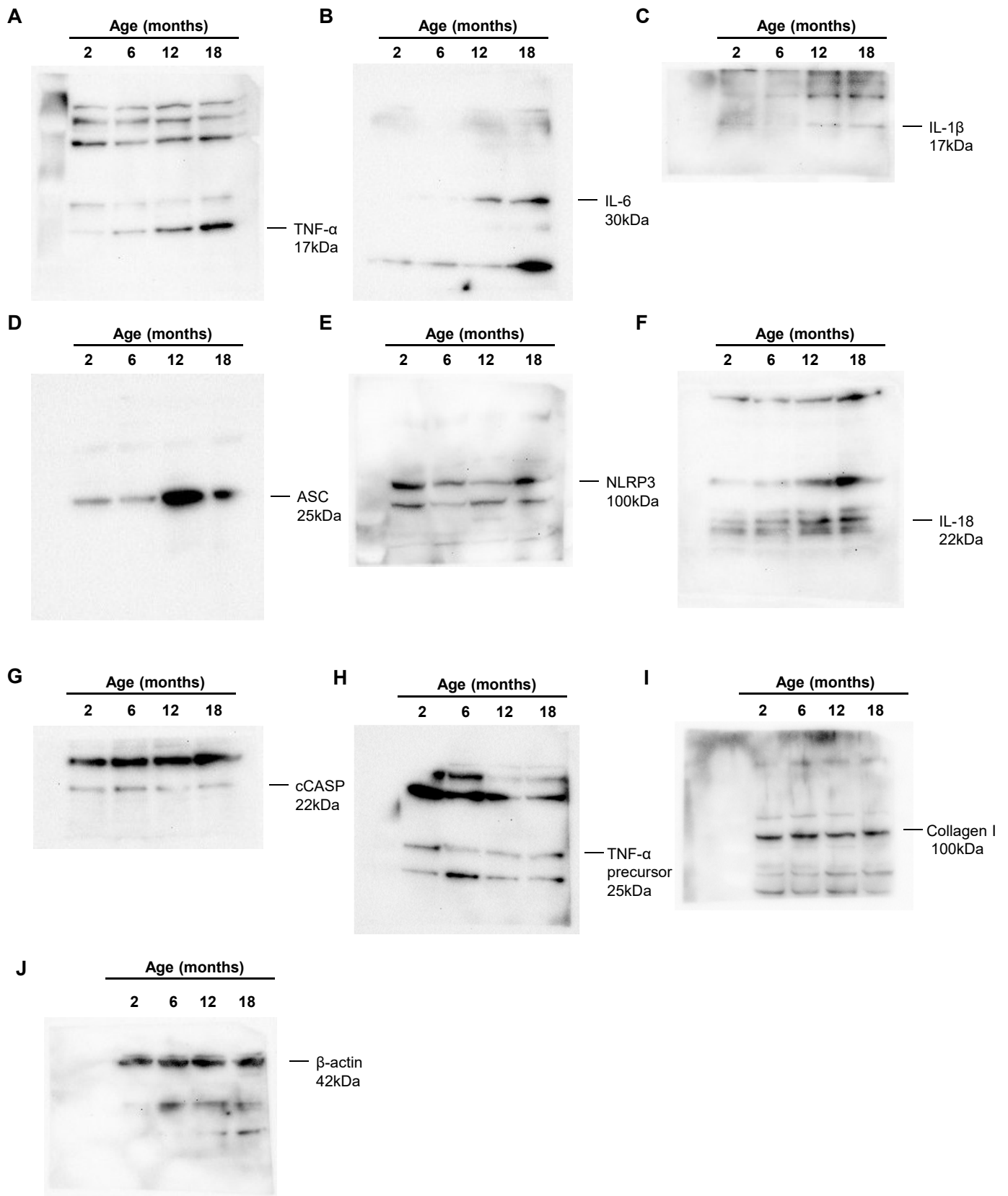
Supplemental Figure 2. Representative dot plots of B cells (A), NK1.1+TCRβ- NK cells (B), CD11b+ and T cells (C), F4/80+CD11b+ macrophages (D) and CD4+ and CD8+ T cells (E) identified in ovaries from 2, 6, 12 and 18-month-old mice by Flow cytometry. n = 3-5 per cohort.

Supplemental Figure 3



Supplemental Figure 3. Number of B cells (A), T cells (B), CD4+ (C) and CD8+ T cells (D), NK1.1+TCRβ- NK cells (E), CD11b+ cells (F) and F4/80+CD11b+ macrophages (G) identified in ovaries from 2, 6, 12 and 18-month-old mice. n = 3-5 per cohort. Data are presented as mean±SEM; A, B, E, F and G: Kruskal-Wallis test, Dunn's multiple comparisons test (p>0.05); C and D: Ordinary One-way ANOVA, Tukey's multiple comparisons test: a and b are significantly different among groups (p<0.05).

Supplemental Figure 4



Supplemental Figure 4. Representative images of full length Western blots showing ovarian protein levels of TNF- α (A), IL-6 (B), IL-1 β (C), ASC (D), NLRP3 (E), IL-18 (F), cleaved Casp1 (G), TNF- α precursor (H), collagen I (I) and β -actin (J) from 2, 6, 12 and 18-month-old mice.

Supplemental Table 1. Stage of estrous cycle

Mice age (Months)	Stage of estrous cycle
2	Estrus
	Estrus
	Estrus
	Estrus
	Diestrus
6	Diestrus
	Diestrus/Proestrus
	Diestrus
	Diestrus
12	Diestrus
	Diestrus
	Diestrus
18	Metestrus
	Diestrus
	Estrus

Supplemental Table 2. List of primers used for quantitative real-time PCR

Gene	Sequence (5'→3')	PCR product (bp)
<i>Asc</i> (PYD and CARD domain containing)	F: GACAGTACCAGGCAGTTCGT	96
	R: AGTCCTTGCAAGTCAGGTTTC	
<i>Gapdh</i> (glyceraldehyde-3-phosphate dehydrogenase)	F: TCCATGACAACCTTTGGCATTG	72
	R: CAGTCTTCTGGGTGGCAGTGA	
<i>Casp1</i> (Caspase 1)	F: CACGCCCTGTTGGAAAGGAA	94
	R: CCCTCAGGATCTTGTCAGCCA	
<i>Ccl5</i> (Chemokine (C-C motif) ligand 5)	F: TTTGCCTACCTCTCCCTCG	85
	R: CGACTGCAAGATTGGAGCACT	
<i>Col1a1</i> (Collagen, type I, alpha 1)	F: CCGATGGATTCCCGTTCGAG	97
	R: GAGGCCTCGGTGGACATTAG	
<i>Col4a1</i> (Collagen, type IV, alpha 1)	F: GCGTAAGTTCAGCACCATGC	173
	R: CACAAACCGCACACCTGCTA	
<i>Csf1</i> (Colony stimulating factor 1)	F: GAACAAGGCCTGTGTCGGAA	161
	R: CCACATCTCGGCTAGAGCAC	
<i>Csf2</i> (Colony-stimulating factor 2)	F: AACTCCGAAACGGACTGTG	145
	R: AAGCTGGATTCAGAGCTGGC	
<i>Il10</i> (Interleukin 10)	F: GCTCTTACTGACTGGCATGAG	105
	R: CGCAGCTCTAGGAGCATGTG	
<i>Il18</i> (Interleukin 18)	F: CCTTTGAGGCATCCAGGACA	87
	R: GGGAAACAGCCAGTGTTCAGT	
<i>Il1a</i> (Interleukin 1 alpha)	F: GTATGCCTACTCGTCGGGAG	120
	R: GGCAACTCCTTCAGCAACAC	
<i>Il1b</i> (Interleukin 1 beta)	F: GAAGAAGAGCCCATCCTCTG	91
	R: GGAGCCTGTAGTGCAGTTGT	
<i>Il6</i> (Interleukin 6)	F: CTGCAAGAGACTTCCATCCAG	131
	R: AGTGGTATAGACAGGTCTGTTGG	
<i>Nlrp3</i> (NLR family, pyrin domain containing 3)	F: CAGAGCCTACAGTTGGGTGA	193
	R: TAGCAGTGAAGAGCAGTGCG	
<i>Tnfa</i> (Tumor necrosis factor alpha)	F: CCTGGCCTCTTACCTTGTG	100
	R: AGCCTGGTCACCAAATCAGC	

Supplemental Table 3. Panel of antibodies and other stains used for Flow Cytometry

Antibody	Conjugate	Clone	Specificity	Concentration	Company
CD19	BV650	1D3	B cells	1:400	BD Biosciences, 563235
CD4	BUV395	GK1.5	CD4 ⁺ T cells	1:400	BD Biosciences, 563790
CD8a	PerCP-Cy5.5	53-6.7	CD8 ⁺ T cells	1:400	BD Biosciences, 551162
CD11b	FITC	M1/70	CD11b ⁺ cells	1:400	BD Biosciences, 557396
F4/80	PE	T45-2342	Macrophages	1:200	BD Biosciences, 565410
NK1.1	PE-Cy7	PK136	NK cells	1:600	BD Biosciences, 552878
TCR β	BV510	H57-597	T cells	1:400	BD Biosciences, 563221
Live/Dead Viability Stain 700	FVS700	-	Dead cells	1:2000	BD Biosciences, 564997