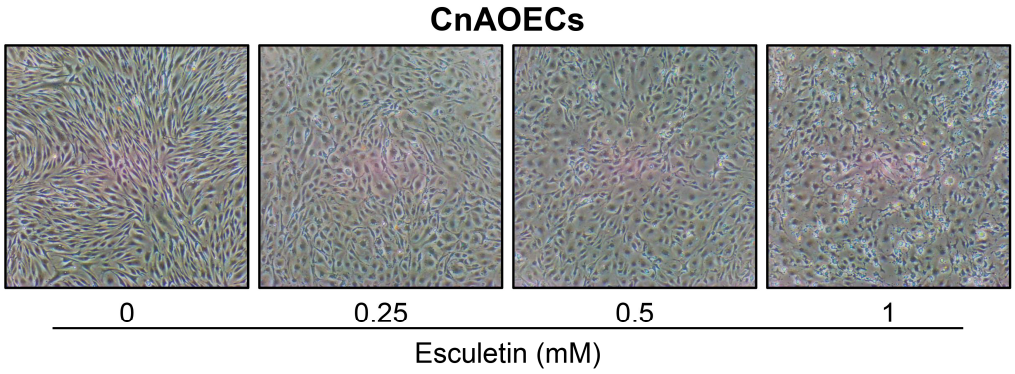
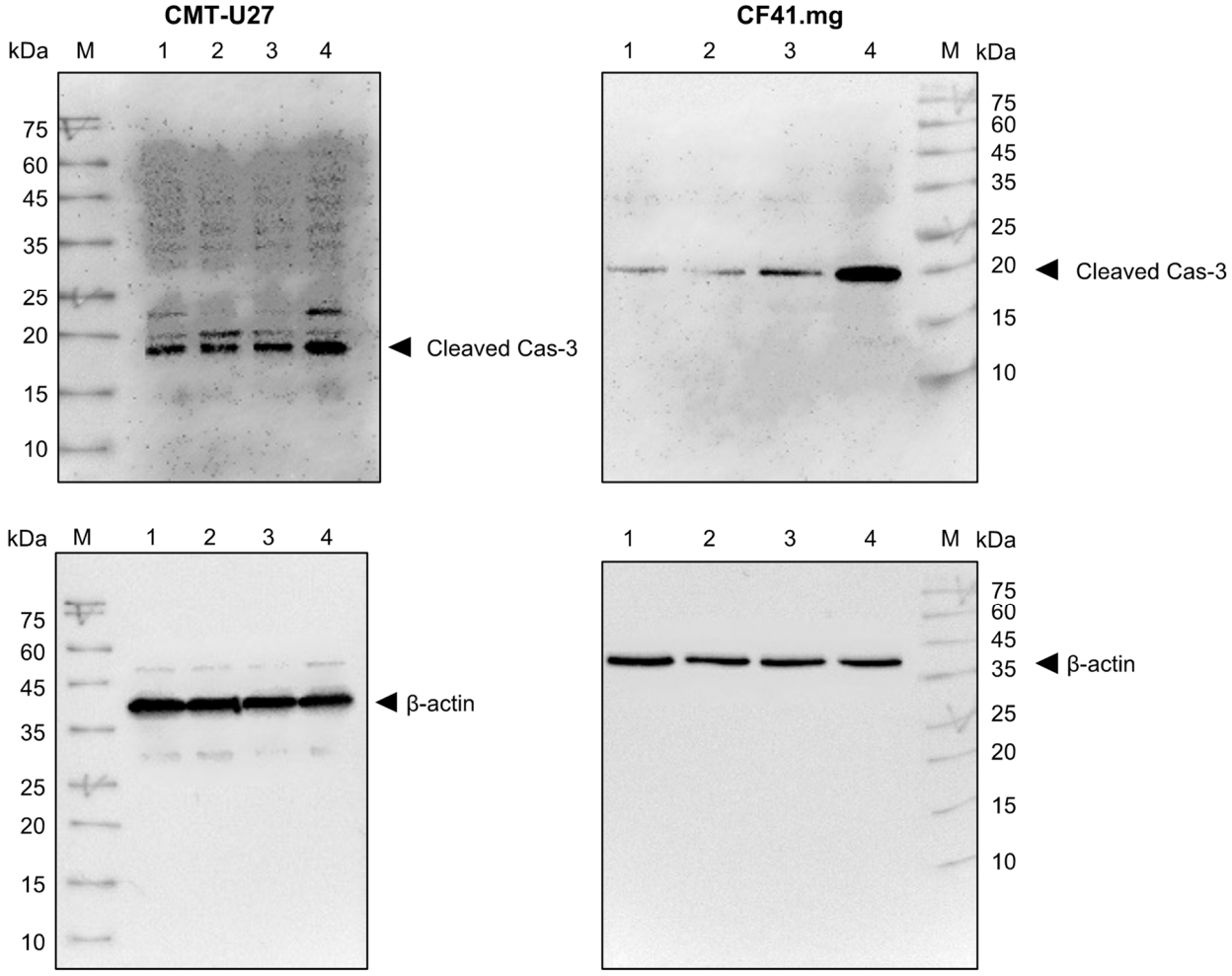


Figure S1. The effect of esculetin on cell viability in canine aortic endothelial cells. Canine aortic endothelial cells (CnAOECs) were treated with 0, 0.25, 0.5, and 1 mM of esculetin for 24 h. Following treatment, the cell images were photographed using a microscope (Nikon Eclipse TS100; Nikon Corporation, Tokyo, Japan).

a



Lane M: Molecular weight markers
Lane 1: Untreated
Lane 2: Esculetin (0.25 mM)
Lane 3: Esculetin (0.5 mM)
Lane 4: Esculetin (1 mM)

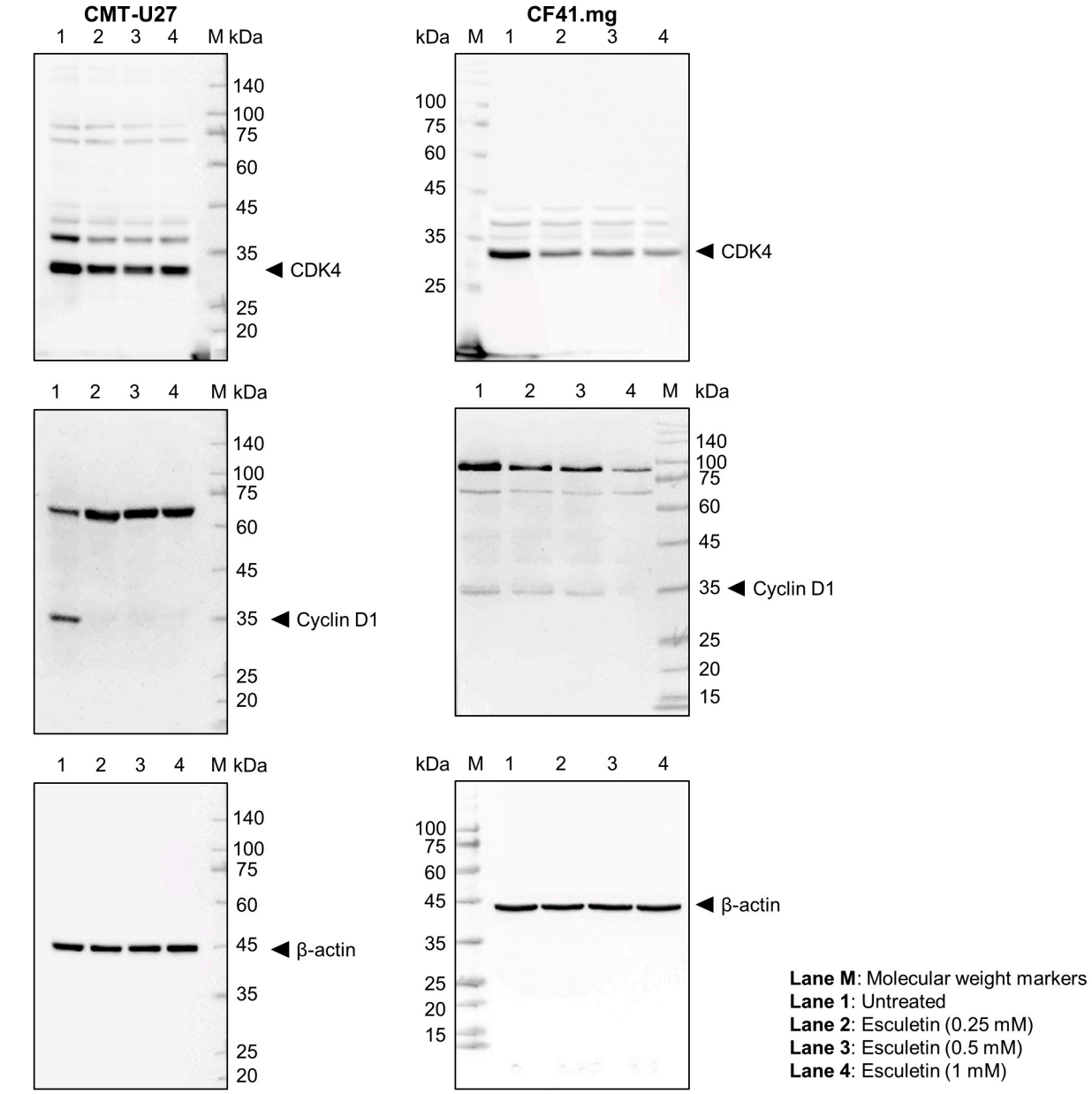
b

CMT-U27	
Esculetin (mM)	**Cleaved Cas-3/β-actin**
0	1.00 ± 0.04
0.25	1.23 ± 0.06
0.5	1.31 ± 0.02
1	1.68 ± 0.13

CF41.mg	
Esculetin (mM)	**Cleaved Cas-3/β-actin**
0	1.00 ± 0.18
0.25	1.34 ± 0.24
0.5	1.68 ± 0.21
1	5.00 ± 0.72

Figure S2. Immunoblots and densitometry reading/intensity ratio of apoptotic marker in CMT cells. CMT-U27 and CF41.mg cells were treated with 0, 0.25, 0.5, and 1 mM of esculetin for 24 h or 48 h. The protein expression of cleaved cas-3 (17/19 kDa) was analyzed by (a) western blots and (b) densitometry reading/intensity ratio. The band intensity was normalized to the corresponding β-actin value. Cas-3, caspase-3.

a



b

CMT-U27		
Esculetin (mM)	**CDK4/β-actin**	**Cyclin D1/β-actin**
0	1.00 ± 0.05	1.00 ± 0.12
0.25	0.52 ± 0.01	0.58 ± 0.03
0.5	0.39 ± 0.01	0.68 ± 0.04
1	0.40 ± 0.03	0.60 ± 0.04

CF41.mg		
Esculetin (mM)	**CDK4/β-actin**	**Cyclin D1/β-actin**
0	1.00 ± 0.07	1.00 ± 0.11
0.25	0.46 ± 0.02	0.81 ± 0.04
0.5	0.40 ± 0.02	0.75 ± 0.01
1	0.33 ± 0.03	0.69 ± 0.06

Figure S3. Immunoblots and densitometry reading/intensity ratio of cell cycle related proteins in CMT cells. CMT-U27 and CF41.mg cells were treated with 0, 0.25, 0.5, and 1 mM of esculetin for 24 h or 48 h. The protein expression of CDK4 (34 kDa) and cyclin D1 (35 kDa) was analyzed by (a) western blots and (b) densitometry reading/intensity ratio. The band intensity was normalized to the corresponding β-actin value.