

## **Supplementary methods**

### **Follow-up**

The clinical 2-year follow-up was obtained from veterinary practitioners, as well as owners when needed (for the precise date of death). Disease-free interval (DFI) was defined as the interval from mastectomy to the earliest local recurrence, new primary tumor, lymph node and/or distant metastasis. For overall survival (OS, time between mastectomy and death from any cause), censored cases corresponded to alive animals, and uncensored cases to dead animals, whatever the cause of death. For cancer-specific survival (SS, time between mastectomy and death attributable to the mammary carcinoma), uncensored cases corresponded to cats dead from cancer, and censored cases to live cats, cats dead from unknown causes, and cats dead from a cause unrelated to cancer.

### **Immunohistochemistry**

The following immunohistochemical protocols were applied: p63 (murine clone 4A4, abcam ab735, heat-induced epitope retrieval (HIER) for 30 minutes in CC1 medium, pH 8.5, Ventana Medical Systems, incubation time 30 minutes at 37°C,

1:100), cytokeratins (murine clones AE1-AE3, Dako M3515, HIER for 8 minutes in CC1 and for 8 minutes using protease-1, Ventana Medical Systems, incubation time 44 minutes at 37°C, 1:200), LMO2 (rabbit clone SP51, Spring M351, HIER for 48 minutes in CC1, incubation time 1 hour and 32 minutes at 37°C, 1:50), ER alpha (murine clone C311, Santa Cruz Biotechnology sc-787, no antigen retrieval, incubation time 44 minutes at 37°C, 1:50), PR (murine clone 10A9, Meridian Life Science K42546M, HIER for 1 hour in CC1, incubation time 1 hour and 40 minutes at 37°C, 1:50), HER2 (rabbit clone 4B5, Roche Diagnostics 790-2991, HIER for 30 minutes in CC1, incubation time 8 minutes at 37°C, prediluted), the proliferation marker Ki-67 (murine clone MIB1, Dako M7240, HIER for 1 hour in CC1, incubation time 32 minutes at 37°C, 1:50), cytokeratin 14 (CK14, murine clone LL002, Santa Cruz Biotechnology sc-58724, HIER for 1 hour in CC1, incubation time 44 minutes at 37°C, 1:150), and FoxP3 (rabbit clone SP97, Spring M3970, HIER for 32 minutes in CC1, incubation time 32 minutes at 37°C, 1:150). The detection systems were the iView DAB detection kit for ER, PR, and Ki-67 (Ventana Medical Systems, 760-091), chosen to minimize background staining; the UltraView Universal DAB detection kit for HER2 (Ventana Medical Systems, 760-500), as recommended by the manufacturer; and for p63, cytokeratins, LMO2, and FoxP3, the OptiView DAB IHC

37 Detection Kit (Ventana Medical Systems, 760-700), a 3-step indirect biotin-free  
38 system, chosen for its high sensitivity, and absence of background staining in feline  
39 tissues. The three detection systems use horseradish peroxidase with hydrogen  
40 peroxide substrate, and 3,3'-diaminobenzidine tetrahydrochloride (DAB) as  
41 chromogen.