

1 Supplementary Material S1

2 Determination of calretinin

3 Concentrations of calretinin in plasma were determined using the Calretinin ELISA kit by DLD
4 Diagnostika GmbH (Hamburg, Germany) according to the manufacturer's instructions. All
5 reagents and samples were equilibrated to 22 °C and the incubations were performed at 22
6 °C. Plasma samples (30 µl) were diluted 1:5 in the dilution buffer. All samples were
7 determined in duplicate. Optical densities were measured using a SpectraMax 384 plus plate
8 reader (Molecular Devices, Sunnyvale, CA, USA). The standard curve was obtained by four-
9 parameter curve fitting using SoftMax Pro 4.7.1 (Molecular Devices).

10

11 Determination of mesothelin

12 Concentrations of mesothelin in plasma samples were determined using the ELISA kit
13 MESOMARK by Fujirebio Diagnostics, Inc. (Malvern, PA, USA) according to the
14 manufacturer's instructions with modifications as described before (39).

15

16 Statistical analyses

17 Calretinin and mesothelin concentrations were presented as box plots with median and
18 interquartile range (IQR), overlaid with dot plots. Whiskers represent minimum and
19 maximum. The two-sample Wilcoxon rank-sum test was applied to compare the distribution
20 of calretinin and mesothelin values between prediagnostic mesothelioma cases and
21 controls.

22 Biomarker classification performance was determined by receiver operating curve (ROC)
23 with the area under curve (AUC) estimated to assess a marker's sensitivity for varying values
24 of specificity. 95% Wald confidence intervals (CI) were calculated for the AUCs. For the

25 sequential combination of both markers, first calretinin was used for classification.
26 Afterwards, mesothelin was examined for calretinin-negative subjects only. This procedure
27 was repeated for all possible cut-points to calculate the related sensitivities and specificities
28 resulting in a point cloud instead of a traditional ROC curve. Therefore, the AUC for this
29 combination was calculated as descriptive measure for the “best case” scenario, so that the
30 points with highest specificities and sensitivities were considered. The related intervals show
31 minimum and maximum obtainable AUCs.
32 Statistical analyses were performed using SAS/STAT and SAS/IML software, version 9.4 (SAS
33 Institute Inc., Cary, NC, USA) and GraphPad Prism version 9.5.1 (GraphPad Software, La Jolla
34 California, USA) was used for generating graphs.