CORRIGENDUM

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Corrigendum: CEACAM1 regulates TIM–3–mediated tolerance and exhaustion

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In this Letter, we published the crystal structure of a heterodimer of the human (h)CEACAM1 IgV domain and hTIM-3 IgV domain (Protein Data Bank (PDB) accession 4QYC). Since publication, E. Sundberg and S. Almo have questioned our model, and stated that they had obtained better results refining a hCEACAM1–hCEACAM1 homodimer model against our diffracted amplitudes. We confirm that a homodimer model indeed fits our crystallographic data better, as judged by most statistical measures (see Supplementary Table 1). We have therefore withdrawn the deposited heterodimer model (PDB code 4QYC) from the PDB, and replaced it with a more accurate homodimer model (PDB code 5DZL). We thank E. Sundberg and S. Almo for bringing this to our attention, and apologize for any confusion the original structure may have caused.

Our error was rooted in an assumption, which now seems to be invalid, that a crystal of a chimaeric two-domain protein should give X-ray diffraction data reflecting both domains. We sought evidence for the hTIM-3 domain in our data using multiple molecular replacement and crystallographic strategies, and also reprocessed the data using lower symmetry space groups in case the hTIM-3 signal was lost in the computational averaging. We now believe that the relatively low resolution of the dataset (3.4 Å) and the similarities between the folds of hCEACAM1 and hTIM-3 make it impossible to model the hTIM-3 IgV domain confidently using the data at hand. To understand how dimeric hCEACAM1 could predominate in a crystal built from a protein construct designed to ensure a 1:1 ratio of hCEACAM1 and hTIM-3, we performed western blot analyses on the materials used for crystallization, which showed a predominant species of ~26 kDa, consistent with intact chimaeric protein and minor additional lower molecular mass species, suggesting proteolysis as the reason for the absence of hTIM-3, consistent with the long time (months) required for crystal growth (Supplementary Fig. 1). Considering the strong tendency of CEACAM1 to crystallize as dimers, even a small amount of free homodimer could have preferentially crystallized.

In light of this, it is important to consider whether the incorrect structure calls into question any of the other results and conclusions of the paper. Presumably, the chimaeric protein used in our Letter was unaffected because the conditions did not favour proteolysis. Nevertheless, we felt obliged to extend our biophysical experiments to provide additional support for a direct interaction between hCEACAM1 and hTIM-3. Co-crystallization attempts with hCEACAM1 and hTIM-3 would be hindered by the tendency of CEACAM1 to homodimerize with a dissociation constant (K_d) of 450 nM (ref. 1). We instead pursued NMR and surface plasmon resonance (SPR) studies using purified tag-free IgV domains of hTIM-3 and hCEACAM1 (Supplementary Methods and Supplementary Fig. 2a–c). NMR ^{15}N-HSQC spectra of ^{15}N-labelled hCEACAM1 IgV domains showed spectral changes after incubation with unlabelled hTIM-3 IgV in the presence of 2 mM calcium (Supplementary Fig. 3a), which binds to the FG loop of hTIM-3 IgV (Supplementary Fig. 3b); calcium alone did not induce spectral changes in the ^{15}N-labelled hCEACAM1 ^{15}N-HSQC spectra (Supplementary Fig. 2d). So far, hCEACAM1 dimerization and oligomerization at experimental concentrations for NMR prevent us from mapping the spectral changes to the putative hTIM-3 binding site on the GFCC′ face of hCEACAM1. We also performed SPR experiments in which we immobilized hCEACAM1 IgV and flowed over hTIM-3 IgV plus calcium at varying concentrations. In the initial SPR experiments, 250 response units (RU) of hCEACAM1 IgV protein were directly immobilized via amine coupling and resulted in low levels of the hTIM-3 receptive surface ($B_{max} < 10\%$), probably due to misoriented and dimeric immobilized hCEACAM1. Global fit analysis of the hTIM-3 binding sensorgrams to a 1:1 Langmuir binding model (Supplementary Fig. 3c, top) yielded an underestimated association rate, an overestimated slow disassociation rate, and an overall K_d value of 2.2 μM (Supplementary Table 2). Further SPR studies used an oriented strategy in which C-terminal biotinylated hCEACAM1 IgV was immobilized at predominantly monomeric concentrations to a neutravidin-coupled biosensor chip, allowing CEACAM1 N-termini to be directed toward the solution. Single cycle kinetic studies were performed with serial hTIM-3 concentrations and both improved kinetic fit analysis and extrapolated steady-state analysis were performed using a 1:1 Langmuir binding model (Supplementary Fig. 3d and Supplementary Table 2), which yielded similar affinity constants (2–6 μM) to that obtained with the amine coupling experiments.

In conclusion, our new solution-based NMR and surface-based SPR studies provide further independent biophysical evidence to support a direct interaction between hCEACAM1 and hTIM-3 via their N-terminal IgV domains. After withdrawal of our crystallographic model, we cannot confidently state the stoichiometry, describe the molecular details, or differentiate between *cis*/*trans* modes of IgV domain interaction, as claimed in Letter. Future studies are needed to determine whether the interaction may be further facilitated by additional factors or by higher order oligomerization of hCEACAM1.

We also provide a corrected version of Fig. 2i (as Supplementary Fig. 3e to this Corrigendum), which during the review process inadvertently duplicated the left panel of the autoradiogram.

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Supplementary Information is available in the online version of the Corrigendum.

1. Bonsor, D., Gunther, S., Beadenkopf, R., Beckett, D. & Sundberg, E. Diverse oligomeric states of CEACAM IgV domains. *Proc. Natl Acad. Sci. USA* **112,** 13561–13566 (2015).